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Monocarboxylate transporters and heat shock proteins in domestic pigs in relation to stress and meat quality

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Academic Dissertation

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Contents

Abstract

In stressful situations, anaerobic muscles of the domestic pig easily produce lactate. Lactate is transported out of the muscles by monocarboxylate transporters (MCTs), which are important proteins in muscle pH regulation. The immunoblot technique was used in Finnish Landrace and Yorkshire pigs to investigate the amounts and isoform distributions of MCT1, MCT2 and MCT4 in five different skeletal muscles; oxidative *M. masseter* (M) and *M. infraspinatus* (IS), and glycolytic *M. longissimus dorsi* (LD), *M. semimembranosus* (S) and *M. gluteus superficialis* (G) were used. Oxidative capacity, measured by citrate synthase and 3-hydroxyacyl-CoA dehydrogenase activities, was highest in M and IS, and low in LD, S and G. In all muscles, the amount of MCT1 was small in comparison to that found in other species and the inter-individual variation was high. MCT2 was more abundant in glycolytic than oxidative muscles, while MCT4 was found in equal amounts in all muscles. The results, together with measured concentrations of lactate, suggest that MCT2 may function as the housekeeping lactate transporter, preventing acidification, especially of highly glycolytic muscles. The results also support the view that, similar to in other species, MCT4 in pigs may be important at high lactate concentrations. No correlation between MCTs and meat quality (drip loss, colour and pH) in the above-mentioned muscles was found.

MCTs also facilitate the absorption of lactate in the small intestine and short-chain fatty acids in the colon. Here, the amounts of MCT1, MCT2, MCT4 and CD147, a chaperone for MCT1 and MCT4, were measured from the small intestine (2.5 m from pylorus) and proximal colon by immunoblotting. MCT1 and MCT4 were found in both sites, but MCT2 only in the small intestine. In both the small intestine and colon, Yorkshire pigs had more CD147 than Landrace pigs, while no interbreed differences were found in MCT isoforms. Since CD147 is essential for the activity of MCT1 and MCT4, the breed difference suggests that the MCT activity is higher in Yorkshire than in Landrace pigs. Further studies are needed to establish the physiological significance of this finding.

Stress has a significant influence on the physiology of the intestinal epithelium. Cells respond to stress by synthesizing heat shock proteins (HSPs), especially HSP72. In this study, HSP72 concentrations were measured in the porcine small intestine, colon and serum. HSP72 in the colon correlated with serum HSP72, suggesting that the colon is a significant source of serum HSP72 and serum HSP72 may reflect changes in the permeability of intestinal epithelium due to stress. Colon HSP72 content was negatively correlated with carcass weight (growth). HSP72 in serum showed no correlation with such conventional markers of stress, as blood lactate or serum concentrations of cortisol, glucose, free fatty acids or haptoglobin.

Polymorphisms in the promoter area of the *hsp70.2* gene have earlier been suggested to affect expression of HSP72. We found six different single-nucleotide polymorphisms and a novel nonanucleotide insert polymorphism (GGAGAGATC) at 11 bp upstream of the start site of translation. Only the T/C mutation at -118 bp from the translation start site had a significant impact on HSP72 protein content in LD muscle. However, these polymorphisms were shown to have some effects on meat quality traits. The most interesting finding was that in Landrace pigs drip loss was greater in pigs with the insert (-11) than in normal pigs, thus, this character could also have commercial significance.

Measuring stress in animals is a challenging task. A continuous production of small amounts of lactate occurs in the white muscles of the domestic pig, to which the animal seems partially adapted because of expression of the high-affinity isoform, MCT2, in their skeletal muscle. None of the MCT isoforms, however, is useful when the level of stress needs to be measured. The wide interindividual variation in the amounts of stress-synthesized HSP72 suggests that it may have potential as a marker of stress in pigs, but more studies are needed to confirm this.

List of original publications

This thesis is based on the following original publications, which are referred to in the text by their numbers:

1) Sepponen K., Koho N., Puolanne E., Ruusunen M. and Pösö A.R. (2003) Distribution of monocarboxylate transporter isoforms MCT1, MCT2 and MCT4 in porcine muscles. Acta Physiologica Scandinavica 177: 79-86.

2) Sepponen K., Ruusunen M., Pakkanen J.A. and Pösö A.R. (2007) Expression of CD147 and monocarboxylate transporters MCT1, MCT2 and MCT4 in porcine small intestine and colon. The Veterinary Journal. 174: 122-128.

3) Sepponen K. and Pösö A.R. (2006) The inducible form of heat shock protein 70 in the serum, colon and small intestine of the pig: comparison to conventional stress markers. The Veterinary Journal 171: 519-524.

4) Sepponen K., Honkavaara M., Pösö A.R., Ruusunen M. and Reeben M. Polymorphisms in *hsp70.2* promoter region of Finnish Landrace and Yorkshire pigs. Submitted.

Papers 1-3 are included in this dissertation as reprints with permission from journals concerned. In addition, some unpublished material has been presented.

Abbreviations

a* value	redness of meat
APP	acute phase protein
b* value	yellowness of meat
bp	base pair
CS	citrate synthase
EP	experimental pig
FFA	free fatty acid
G	*Musculus gluteus superficialis*
GP	glycolytic potential
HAD	3-hydroxyacyl-CoA dehydrogenase
HRP	horseradish peroxidase
HSE	heat shock element
HSF	heat shock transcription factor
HSP	heat shock protein
IS	*Musculus infraspinatus*
I-FABP	intestinal fatty acid binding protein
K_m	Michaels constant, equal to the substrate concentration at which the reaction rate of the enzyme is half its maximal value
L* value	lightness of meat
LD	*Musculus longissimus dorsi*
LDH	lactate dehydrogenase
M	*Musculus masseter*
MCT	monocarboxylate transporter
mRNA	messenger RNA
PCR	polymerase chain reaction
pH_1	pH value measured 45 min after stunning
pH_u	pH value measured 24 h after stunning
PSE	pale, soft and exudative meat
RBC	red blood cell
S	*M. semimembranosus*
SCFA	short-chain fatty acid
SD	standard deviation
SMCT	sodium monocarboxylate transporter
SNP	single-nucleotide polymorphism
SP	slaughterhouse pig
V_{max}	maximal velocity, equal to the substrate concentration at which the reaction rate of the enzyme is maximal
w.w.	wet weight

1. Introduction

The aim in domestic pig breeding has been to increase growth rate and lean meat content, to improve feed efficiency and to reduce fat content. Today, the growth rate during the fattening period is high, and pigs reach a body weight of approximately 100 kg in 140-170 days (Ruusunen and Puolanne 2004). Simultaneously, breeding has also led to a low oxidative capacity (Essén-Gustavsson 1986, Karlsson 1993, Ruusunen 1994), and also weakness of muscles, bones, joints and connective tissue is already evident (Dammrick 1970, Lucke et al. 1977). Very high growth rate has been shown to be the main reason for high incidence of osteochondrosis in pigs (Reiland 1978). On the other hand an increased incidence of porcine stress syndrome, leading to pale, soft and exudative meat (PSE), was a consequence of selection towards leanness in pigs (Karlsson 1993). Recently, zones of PSE-like meat, characterized by a loose structure, have also been observed in the porcine *M. semimembranosus* (Minvielle et al. 2001, Franck et al. 2002, Voutila et al. 2005). High carcass weight has been shown to be a risk factor for this 'loose-structured' meat (Minvielle et al. 2001).

The oxidative capacity of the domesticated pig is low (Essén-Gustavsson 1986, Karlsson 1993, Ruusunen 1994), and the muscles contain mainly thick fast glycolytic fibres (IIB), which in stressful situations easily switch their energy metabolism to anaerobic glycolysis. Generally, lactate formed in glycolytic (white) muscle fibres is transported to the liver or to oxidative (red) fibres to be used as a fuel. In domestic pigs, however, the transport of lactate out of muscles is limited by poor capillarization (Ruusunen and Puolanne 2004) and its utilization as a fuel is limited by the small number of oxidative fibres (Essén-Gustavsson 1986). Since the mitochondrial density in the white muscles is lower than in red muscles (Beecher et al. 1969), lactate and protons may accumulate in muscles, causing pain in living muscle and, perimortally, quality defects in meat. The muscle fibres protect themselves from the acidification by transporting both lactate and protons out of the cell (Poole and Halestrap 1993). In all species studied, efflux of lactate and protons is facilitated mainly by monocarboxylate transporters (MCTs), which cotransport a proton and a lactate anion through the cell membrane (Juel 1998, Halestrap and Price 1999). Among monocarboxylates, the transport of lactate is quantitatively the most important, but MCTs also transport other monocarboxylates, such as butyrate, acetate and propionate, which are important especially in the gastrointestinal tract (Halestrap and Price 1999). At least 14 different MCT isoforms (MCT1-MCT14) have been identified thus far, MCT1, MCT2 and MCT4 being the best characterized and known to transport lactate (Halestrap and Meredith 2004). These three isoforms have different species-, and tissue- specific distributions (Halestrap and Price 1999). The low oxidative capacity, sparse capillarization and fibre type distribution of the muscles of the domestic pig render it an interesting animal for studies of MCT isoform composition. The exact MCT isoform pattern in porcine muscles, whether interindividual variation exists between pigs and whether MCTs show any correlation with meat quality or other physiological parameters, are unknown.

Commercial pig production makes it difficult for animals to follow their natural behaviour (Lindfors et al. 2005). The animals are kept under barren conditions, in large groups with high stocking densities, where physical activity is very restricted. As mentioned above, the highly anaerobic muscles of domestic pigs are easily acidified due to accumulation of lactate and protons. At the cellular level, this acidification of the muscle during stress triggers a stress reaction, which leads to the synthesis of special stress proteins, heat shock proteins (HSPs, Weitzel et al. 1985). HSPs help other proteins to maintain their conformation and even assist in their repair (Hendrick and Hartl 1993, Kiang and Tsokos 1998). HSPs form a protein family classified according to their

molecular weights (Locke 1997, Liu and Steinacker 2001). One of the most abundant and best-characterized is the 70 kDa family (HSP70), which contains both inducible (HSP72) and cognate (HSP73) forms (Kiang and Tsokos 1998). In addition to acidosis, HSP72 expression is known to be activated by many pathological and environmental factors. Trauma (Pittet et al. 2002), vascular disease (Wright et al. 2000), hyperthermia (Currie and White 1983, Flanagan et al. 1995), exercise (Walsh et al. 2001, Febbraio et al. 2002, Pösö et al. 2002), age (Rea et al. 2001) and even psychological stress (Fukudo et al. 1997, Isosaki and Nakashima 1998) have been shown to increase HSP72 mRNA or protein concentrations. The time pattern of HSP induction varies from species to species, but because the synthesis of new proteins is required, HSPs do not respond to stress as rapidly as traditional stress markers. As an exception, serum HSP concentration may increase rapidly, when, during necrotic cell death or tissue injury, earlier synthesized HSPs are released from tissue into the serum (Kimura et al. 2004). In this study, it was examined whether stress-inducible HSP72 could be considered a marker of stress and whether the polymorphisms in the HSP72 promoter area cause variation in HSP72 expression. We chose to study HSP72 expression in intestines, which undergo acidification during stress and are highly stress-sensitive organs (Nabuurs et al. 2001a, Söderholm and Perdue 2001).

The domestic pig is a very interesting animal for research because it is highly inactive and its physiology has undergone changes as a result of decades of breeding. The aim in this study was to measure the distribution of MCT isoforms and their amounts in the muscles of domestic pigs, where most muscle fibres are white and oxidative capacity is poor. A further aim was to compare these muscles with each other to uncover any differences between individuals, and to determine whether a correlation exists between MCT proteins and meat quality. If lactate and protons are not transported efficiently out of cells, animals experience stress caused by acidification. The final aim was to investigate stress-induced HSP72 in porcine tissues in the attempt to find a new reliable marker for stress in pigs.

2. Review of the literature

2.1 Porcine physiology

The heart of the domestic pig is small relative to its body weight. Compared with the wild pig, the modern pig has lower blood haemoglobin concentration and lower plasma and blood volume-to-body weight ratios (Yang and Lin 1997). In addition, poor blood circulation and sparse capillarization (Ruusunen and Puolanne 2004) may lead to a shortage of oxygen, transport of fuel metabolites and heat production.

The oxidative capacity of the muscle fibres of the domestic pig is poor (Essén-Gustavsson 1986, Karlsson 1993, Ruusunen 1994, Suuronen 1995); the percentage of white (fast glycolytic, IIB) fibres is high, and these fibres have a large cross-sectional area, a small number of mitochondria and a low capillary density (Ruusunen and Puolanne 2004). Even during moderate physical stress, porcine muscles produce energy through anaerobic metabolism (Essén-Gustavsson 1986). The reason underlying these muscle fibre characteristics in domestic pigs has been speculated to be the breeding of animals for rapid growth and large muscle mass (Kiessling et al. 1982, Lefaucheur et al. 2002), which may favour a high proportion of light, anaerobic fast glycolytic muscle fibres (Kiessling et al. 1982, Lefaucheur et al. 2002) with a large cross-sectional area (Essén-Gustavsson 1986). The proportion of fast glycolytic fibres is 80–90% in *M. longissimus dorsi* (LD) (Essén-Gustavsson et al. 1988, Karlsson 1993, Ruusunen 1994), 50–60% in *M. semimembranosus* (S) (Solomon et al. 1994) and about 60% in the *M. gluteus superficialis* (G) (Bee et al. 1999). Ruusunen and Puolanne (2004) have reported that the area percentages of fast glycolytic fibres in all of these muscles are about 90% and the area percentage of slow oxidative fibres in the same muscles is only about 7%. Compared with domestic pigs, wild pigs have higher percentages of slow oxidative and fast oxidative fibres and lower percentages of fast glycolytic fibres (Bader 1983). Moreover the capillary density in light muscles of wild pigs is twice that of domestic pigs (Ruusunen and Puolanne 2004).

As in other species, fibre type distribution in the muscles of domestic pigs is strongly genetically determined, but physical activity also has an impact on muscle characteristics. The strength of the genetic component is clearly demonstrated in the study of Szentkuti and Schlegel (1981), where despite intensive physical exercise of domestic pigs, these pigs seemed to have a smaller proportion of fast oxidative fibres and a larger proportion of fast glycolytic fibres than wild pigs kept in a pen to restrict their physical activity. In general, the muscles of actively moving animals, such as those of horses, are well capillarized and the oxidative capacity of muscle fibres is high, unlike the muscles of inactive animals, such as those of domestic pigs (Essén-Gustavsson 1986, Karlström 1995). Furthermore in pigs, endurance training has been shown to increase the area of oxidative fibres (Lindholm et al. 1979), but not to alter the fibre type composition of *biceps femoris*, *gracilis* or LD muscles (Fitts et al. 1973, Essén-Gustavsson and Jensen-Waern 1993). Several studies have also demonstrated activities of citrate synthase (CS), 3-hydroxyacyl-CoA dehydrogenase (HAD) and lactate dehydrogenase (LDH) (Jorgensen and Hyldgaard-Jensen 1975) in porcine muscle that show similar adaptive changes as those seen in man and other animals, although adaptations in specific muscles differ between swine and other species (Doizé et al. 1989, McAllister 1997, Petersen et al. 1997). Meat quality traits of treadmill-trained pigs have been measured by Petersen et al. (1997). They found that endurance training significantly increased the content of haem pigments in the *psoas major* muscle but not significantly in the other muscles studied. Meat texture and pH were unaffected. Apparently, the muscle studied and the intensity of the exercise determine the effectiveness of exercise on biochemical characteristics of the muscle.

During exercise or excitement blood pressure, cardiac output and vascular resistance in the splanchnic area are increased (McKiman et al. 1991). The latter decreases blood supply to the splanchnic area and results in an increased blood supply to muscles. The gastrointestinal system is then susceptible to ischaemia, especially in fed animals. The domestic pig may be particularly vulnerable to gastrointestinal ischaemia since its cardiovascular system is mismatched to its body weight (Niewold et al. 2000). In the fattening pig, the intestines develop at a slower rate relative to body weight and body composition (Doornenbal and Tong 1981). However, rapid growth requires high food intake, leading to the gastrointestinal system being burdened with large amounts of food. The intestinal digestive tract of a pig needs to perform at a very high level; hence it is stretched to the limit and is vulnerable to injury (Niewold et al. 2000). Transport lasting only 30 min may induce intestinal ischaemia (Nabuurs et al. 2001b).

The physiological characteristics discussed above, developed by the intensive breeding of domestic pigs, makes them particularly susceptible to anaerobic stress and trauma, which may cause both welfare and meat quality problems. The muscles of domestic pigs easily produce lactate in amounts that exceed their capacity to oxidize it, leading to its accumulation in muscles as well as in blood (Karlsson et al. 1994). Especially during transport and pre-slaughter handling, pigs experience stress, leading to breakdown of glycogen and formation of lactate and protons (Karlsson et al. 1994, Wal et al. 1999). Thus, even moderate exertion can be assumed to cause a significant drop in the pH of porcine muscles. To avoid a critical drop in pH, protons must be transferred out of the cells. In meat animals, this is also important for meat quality because carcasses that exhibit rapid pH decline after slaughter have been shown to be of poorer meat quality than carcasses with a slow decrease in pH (Bendall et al. 1963). Major factors that determine the rate of efflux of lactate and protons from muscles are capillarization, blood flow and their transport across the sarcolemma (Juel 1998). Capillarization is low in porcine white muscles (Ruusunen 1994, Karlström 1995), but very little is known about their capacity to transport protons. Formation of protons activates Na^+/H^+ exchange proteins, which carry one H^+ out from the cell and simultaneously one Na^+ into the cell according to the Na^+ gradient (Madshus 1988). In human muscle, this carrier plays a key role in the regulation of cell pH at rest, but during exercise, the main transporters have been shown to be MCT proteins (Juel 1996, Halestrap and Price 1999), which cotransport a proton and a lactate anion out of the cell according to the electrochemical proton gradient. MCTs have been suggested to be the most important pH regulators of the cell (Juel 1996, 1998; Halestrap and Price 1999).

2.2 Monocarboxylate transporters

The MCT family comprises 14 members (MCT1-MCT14), only four (MCT1, MCT2, MCT3 and MCT4) of which have been experimentally demonstrated to catalyse the proton-linked transport of metabolically important monocarboxylates (Halestrap and Meredith 2004). TAT1 (MCT10) has been shown to transport aromatic amino acids (Halestrap and Meredith 2004), and MCT8 to be an active and specific thyroid hormone transporter (Friesema et al. 2003). In addition, MCTs in the epithelium of the small intestine, colon and blood-brain barrier provide routes for many carboxylated pharmaceutical agents (Enerson and Drewes 2003). All MCTs are membrane proteins, with 12 transmembrane regions and cytoplasmic N- and C-terminal ends (Poole et al. 1996, Halestrap and Price 1999). They transport a monocarboxylate anion and a proton together through the cell membrane according to the electrochemical gradients of substrates. Lactate is quantitatively the most abundant monocarboxylate transported by MCTs, but also other monocarboxylates, such as butyrate, acetate, propionate and ketone bodies, are transported (Halestrap and Price 1999). At least MCT1 and MCT4 need a chaperone protein, CD147 (also known as basigin, EMMPRIN, HT7 or OX-47), in muscle (Halestrap and Price 1999, Juel and Halestrap 1999), red blood cells (RBCs, Koho et al. 2002) and the intestines (Buyse et al. 2002). The suggested model of the topology of

CD147 and MCT1 in the plasma membrane is a dimer of CD147 associated with two MCT1 molecules such that the C-terminus of CD147 in the cytosol is close to the C-terminus of its partner CD147 and to the C- and N-termini of an associated MCT1 molecule (Wilson et al. 2002). Koho et al. (2002) have reported that lactate transport via MCT1 in RBCs of horses that express small amounts of CD147 is insignificant compared with horses with larger amounts of CD147 even though the amount of MCT1 is similar in all horses. This result indicates that MCT1 does not function well without CD147. This has been confirmed later in studies with site-specific mutations (Wilson et al. 2005). CD147 belongs to the immunoglobulin superfamily, and in addition to its role as a chaperone of MCTs, it has several other functions, such as induction of matrix metalloproteases (Toole 2003). A co-protein for MCT2 has recently been identified as gp70, a member of the same protein family as CD147 (Wilson et al. 2005).

The distribution of MCT isoforms is species-, and tissue-specific. MCT1 is found in the majority of tissues of all species studied (Halestrap and Meredith 2004). In muscle tissue, it is present in all fibre types, although being more abundant in muscles that have a high percentage of oxidative fibres, and thus, has been suggested to have a major role in influx of lactate for oxidation (McGullagh et al. 1996, Halestrap and Price 1999, Juel and Halestrap 1999). The K_m of MCT1 for lactate is 3.5-10 mM (Bröer et al. 1998, Halestrap and Price 1999), and as mentioned above, it requires a co-protein, CD147, for its correct plasma membrane expression and function (Halestrap and Price 1999, Juel and Halestrap 1999). Recently, MCT1 has been reported to be a target for immunomodulatory compounds used in immunosuppressive therapy. These compounds block the activity of MCT1 and prevent the rapid phase of T cell division necessary for immune response (Murray et al. 2005). The reason for this has been suggested to be the accumulation of lactate in T cells (Murray et al. 2005). Mutations in the MCT1 gene are responsible for subnormal lactate transport in patients with myopathy on exertion (Merezhinskaya et al. 2000).

The other major MCT isoform found in muscles, especially in white, type II muscle fibres, is MCT4 (Wilson et al. 1998, Pilegaard et al. 1999b), which has been speculated to have a primary role in the efflux of lactate from muscles during intense exercise (Wilson et al. 1998, Juel and Halestrap 1999, Manning Fox et al. 2000). MCT4 is expressed widely also in other glycolytic tissues, such as astrocytes, white blood cells and chondrocytes, in addition to in glycolytic muscle fibres (Halestrap and Meredith 2004). This isoform has a high K_m (17-34 mM) for lactate and its V_{max} is also high (Wilson et al. 1998, Bonen et al. 2000, Dimmer et al. 2000, Manning Fox et al. 2000). MCT4 also needs CD147 for its proper function (Halestrap and Price 1999, Juel and Halestrap 1999). In human muscle tissues, interindividual variation in MCT4 content is larger than interindividual variation in MCT1 content (Pilegaard et al. 1999b).

The third isoform that has been found in the muscle tissue is MCT2. It is present in skeletal muscle of man, rats and hamsters (Garcia et al. 1995, Bonen et al. 2006), although not all studies agree with this (Jackson et al. 1997, Price et al. 1998). MCT2 has previously been proposed to be especially species- and tissue-specific (Jackson et al. 1997), but a recent study suggests that it is also widely distributed (Bonen et al. 2006). Besides skeletal muscle, MCT2 has been found in several tissues of rats, mice and hamsters (Garcia et al. 1995, Jackson et al. 1997, Gerhart et al. 1998). In addition, MCT2 is expressed in equine RBCs (Koho et al. 2002). It has a low K_m for lactate (0.7 mM) and may be adapted to transport lactate more efficiently in environments where pH is acidic and rapid transport is required (Garcia et al. 1995, Bröer et al. 1999). The transport via MCT2 is saturable because its V_{max} is low compared with that of MCT1 and MCT4 (Lin et al. 1998, Bröer et al. 1999). Its biological significance has been proposed to be related to modulation of cell pH and volume (Okamura et al. 2001).

Recently, MCT7 was found to be expressed in human but not in rat skeletal muscle (Bonen et al. 2006), but the physiological role of this transporter is presently unknown.

The expression of MCT proteins changes as a result of such physiological factors as exercise training (Bonen et al. 1998, Pilegaard et al. 1999a, Dubouchaud et al. 2000, Green et al. 2002), diet (Leino et al. 2001), obesity (Py et al. 2001), hormones (Buyse et al. 2002, Fanelli et al. 2003, Wang et al. 2003) and various diseases (Knott et al. 1999, Froberg et al. 2001, Jóhannsson et al. 2001), including bacterial infections (Borthakur et al. 2006). Because of the exceptionally large role of anaerobic metabolism in porcine muscles, these muscles may prove useful in investigating MCTs.

In the pig, the expression of MCTs has thus far been studied only in the gastrointestinal tract, where MCT1 was found in the colon (Ritzhaupt et al. 1998a, 1998b). In addition to the pig, MCTs have been investigated in the gastrointestinal tract of humans (Ritzhaupt et al. 1998b, Hadjiagapiou et al. 2000, Stein et al. 2000, Buyse et al. 2002, Cuff et al. 2002, Fishbein et al. 2002), rats (Takanaga et al. 1995, Tamai et al. 1995, Orsenigo et al. 1999, Schröder et al. 2000), reindeer (Koho et al. 2005), sheep (Müller et al. 2002, Kirat et al. 2006) and calves (Kirat et al. 2005).

In the gastrointestinal tract, MCTs are important in the transport of short-chain fatty acids (SCFAs) and lactate (Ritzhaupt et al. 1998a, 1998b, Müller et al. 2002, Koho et al. 2005). SCFAs, such as acetic, propionic, butyric, isobutyric, valeric, isovaleric, 2-methylbutyric, hexanoic and heptanoic acid, are produced in several parts of the gastrointestinal tract by microbial fermentation of dietary fibre. They are weak acids, but because the pH of the gastrointestinal tract, with the exception of the stomach, is nearly neutral, 90-99% of SCFAs are present as anions rather than as free acids. In all mammals examined, acetate is the main SCFA produced. Propionate and butyrate are also present in large concentrations, although their amounts can vary considerably with diet. Commonly, molar ratios of acetate to propionate to butyrate in mammals vary from ~75:15:10 to 40:40:20 (Bergman 1990). In pigs, as in other monogastric species, the main site for SCFA production and absorption is the colon, but some gastric fermentation has also been measured in the cranial segment of the pig stomach, which is non-glandular (Argenzio and Southworth 1974, Bergman 1990). An estimated 60-70% of the energy of the epithelium of the colon is derived from SCFAs, particularly from butyrate (Scheppach et al. 1992). Butyrate also has other important functions in the intestinal epithelium, such as prevention of certain types of colitis (Scheppach 1994), while acetate increases colonic blood flow and enhances ileal motility (Scheppach 1994). In addition, the three main SCFAs acetate, propionate and butyrate stimulate sodium and fluid absorption in the colon and exert proliferative effects on colonocytes (Scheppach 1994). Lactate is also present in the gastrointestinal tract, formed mainly in the stomach, where concentrations of up to 18 mmol/l have been measured in pigs (Argenzio and Southworth 1974). Lactate is mostly absorbed in the small intestine (Argenzio and Southworth 1974). In the gut, the abundance of the MCT protein in the colonic luminal membrane declines during transition from normality to malignancy, which in turn reduces the SCFA required to maintain regulation of normal differentiation and proliferation in the colonic mucosa (Ritzhaupt et al. 1998b). It has been shown in the colon in vivo that a reduction in MCT1 expression, and hence butyrate transport, can lead to a reduction in intracellular butyrate levels (Daly et al. 2005). Decreased butyrate concentration results in dysfunction in the regulation of genes associated with colonic tissue homeostasis and disease prevention (Daly et al. 2005), thus impairing welfare.

The two functions of MCT proteins described above, namely the regulation of cell pH in skeletal muscles and facilitation of the absorption of SCFA in the gastrointestinal tract, can thus be assumed to be important for physiological harmony and also for animal welfare.

2.3 Influence of stress on animal physiology

Environmental factors, such as limited possibilities to move freely (Janssens et al. 1994), prevention of performing natural behaviours (Bracke and Hopster 2006), heat (Moseley et al. 1994) and noise (Otten et al. 2004), together with previously discussed physiological characteristics may cause stress-related symptoms in pigs. In addition to this constant stress, mixing of animals and transport are two common causes of stress in pigs (Nabuurs et al. 2001b). To cope with stress factors animals need a defence mechanism. This mechanism is called an acute stress response, which includes a 'fight or flight' response. The core neuroendocrine systems that activate due to stress factors are the hypothalamic-pituitary-adrenal axis and the sympathetic adrenomedullary system (Sjaastad et al. 2003). In acute, short-term stress, a sudden environmental change leads to immediate activation of the sympathetic nervous system and a release of adrenaline from the adrenal medulla and noradrenaline from nerve endings. These hormones prepare the individual pig to cope with the demands of metabolic, physical and psychological stressors (Selye 1950, Negrão et al. 2000). If the stressor remains, the hypothalamic-pituitary-adrenal axis is also activated, cortisol is released into the blood and adaptation to the stress begins. Severe chronic stress leads to several other hormonal changes and fatigue, eventually even to death (Sjaastad et al. 2003).

Marked individual differences exist in the magnitude and duration of neuroendocrine reactivity in groups of healthy subjects (Negrão et al. 2000). Biochemical and physiological variables that are frequently used to indicate stress include heart rate, body temperature, blood pressure and serum or blood concentrations of catecholamines, cortisol, lactate, glucose, free fatty acids (FFAs) and acute phase proteins (APPs). These variables respond to stress with different amplitudes and time patterns and are more likely to indicate short-term than long-term stress. Most research has focussed on acute stress, with chronic stress receiving less attention.

Adrenaline, produced and released in the adrenal medulla, and noradrenaline, released also from postganglionic sympathetic neurons, are stored in secretory vesicles, released by exocytosis and circulate freely in the blood. The action of catecholamines allows the body to mobilize its resources in ways that are suitable for facing physical and psychological challenges, e.g. in 'fight or flight' situations. The most important actions of catecholamines include increased breakdown of glycogen in the liver, leading to increased plasma concentration of glucose, and increased breakdown of triglycerides in adipose tissue, leading to increased plasma concentration of FFAs. Catecholamines also increase heart rate and contractility, cardiac output and blood pressure, and change vascular resistance so that more blood is pumped through active skeletal muscles and less through abdominal organs. The release of catecholamines is rapid and their plasma half-life is only 1-3 min (Sjaastad et al. 2003). In pigs during restraint stress, plasma adrenaline and noradrenaline have been shown to increase within 0.5 min after the start of restraint and a decrease was seen as early as 2 min later (Roozen et al. 1995). Because catecholamines increase metabolic rate, lactate is formed more easily in the skeletal muscles of the pig. Lactate is an indicator of physical stress and its concentration in blood increases rapidly whenever aerobic capacity of the muscles is exceeded (Jensen-Waern and Nyberg 1993). Plasma FFAs rise somewhat slower, reaching peak levels 3 h after stress in pigs (Spencer 1980).

Cortisol concentrations also increase rapidly due to stress (Spencer 1980, Pösö and Jensen-Waern 1992); in pigs, a 10-min exertion has been shown to double serum cortisol levels compared with rest (Pösö and Jensen-Waern 1992). Circulating cortisol levels show circadian fluctuation, with high levels in the early morning and a decline towards the evening (Bottoms et al. 1972). Chronic stress has been demonstrated to cause hypercortisolaemia, where circadian rhythm of plasma cortisol is attenuated (Janssens et al. 1993), and repeated exposure to stress can elicit a reduced cortisol response (Becker et al. 1985). Urine cortisol level in pigs returns to basal levels within hours after

transportation (Plastow et al. 2005). Furthermore, a stress-susceptible pig may have up to five times faster metabolic clearance of cortisol than normal pigs (Marple and Cassens 1973), which further complicates the use of cortisol as an indicator of anything other than temporary stress. Warriss et al. (1992) have also speculated that cortisol is a measure of psychological rather than physical stress.

APPs, such as haptoglobin, C-reactive protein and serum amyloid A, are mainly secreted due to trauma, inflammation or infection (Petersen et al. 2004). Haptoglobin is a prominent APP in most species investigated and is considered a diagnostically useful measure in pigs (Petersen et al. 2004). In pigs, haptoglobin is a constitutive plasma protein that increases 5- to 8-fold with stimulation and has been the subject of most studies of porcine APP (Eckersall 2004).

Long-term stress has been shown to reduce growth rate and feed intake in rats (Santos et al. 2000). The weight loss induced by chronic stress is only partially mediated by a reduction in food intake (Martí et al. 1994). Signs of chronic stress can also be seen as disturbances in behaviour, the most common of which are tail biting (Schrøder-Petersen and Simonsen 2001) and stereotypical behaviours (Ladewig et al. 1993) causing a release of opioids in the brain (Chronin et al. 1986). In meat animals, avoidance of excessive stress is important because stress, in addition to reduced growth, has a marked impact on meat quality (Gregory 1998).

Stress sensitivity is not the same in all organs and tissues; the gastrointestinal tract is one of the most stress-sensitive organs (Nabuurs et al. 2001a, 2001b, Söderholm and Perdue 2001). As already mentioned, sympathetic tonus decreases blood flow into the gastrointestinal tract region, the result being that motility is attenuated and chyme stays longer in the stomach and intestines. In fast-growing domestic pigs, the cardiovascular system is insufficiently effective to meet the needs of the rapidly increasing body weight. This further diminishes the availability of blood flow, enabling the development of ischaemia (Niewold et al. 2000). Oedema disease, caused by specific strains of *Escherichia coli*, occurs in most parts of the world with intensive pig husbandry (Bertschinger and Nielsen 1993) and is associated with small intestinal acidosis (Nabuurs et al. 2001a). Intestinal acidosis is known to cause mucosal hyperpermability (Salzman et al. 1994), which may facilitate the influx of pathogens into the bloodstream (Bertschinger and Nielsen 1993). In humans, a significant correlation has been revealed between the intensity of chronic stress and the severity and extent of affective gastrointestinal symptomalogy, especially in patients with irritable bowel syndrome (Bennett et al. 1998). In experimental animals, stress has been shown to reactivate colitis (Million et al. 1999, Qiu et al. 1999). Recent evidence suggests that chronic and possibly permanent changes in gut motility and sensitivity develop in enteric smooth muscle in response to stress (Plourde 1999).

2.4 Heat shock proteins

At a cellular level, the stress response includes synthesis of special stress proteins, heat shock proteins. During stress these proteins have been shown to prevent inappropriate protein aggregation, to mediate the transport of proteins for degradation, to help proteins to maintain their conformation and even to assist in their repair (Hendrick and Hartl 1993, Kiang and Tsokos 1998). HSPs form a large protein family consisting of both constitutively expressed and inducible members, which are classified according to their molecular weight (Locke 1997, Liu and Steinacker 2001). One of the most abundant and best characterized is the 70-kDa family (HSP70), containing both inducible (HSP72) and cognate (HSP73, GRP75 and GRP78) forms (Kiang and Tsokos 1998). HSP73 is synthesized in most cells and is only slightly inducible (Sorger and Pelham 1987), whereas HSP72 is expressed in low quantities in unstressed cells and is primarily stress-inducible (Subjeck and Shyy 1986). These forms are present in both the cytoplasm and nucleus of the cell, whereas a third

major form of HSP70 family, GRP78 (previously referred to as BiP), resides within the lumen of the endoplasmic reticulum. The fourth form of the family, GRP75, is found in mitochondria and chloroplasts (Welch 1992). The nomenclature of the HSP70 family is diverse. In this study, HSP72 is used for inducible and HSP73 for constitutive proteins. HSP70 is used when the text refers to either inducible or constitutive forms, and *hsp70.2* refers to the HSP72 gene.

HSP72 expression is known to be activated by many pathological and environmental factors (Kiang and Tsokos 1998), including psychological stress (Fukudo et al. 1997, Isosaki and Nakashima 1998). For example, acidosis (Weitzel et al. 1985), trauma (Pittet et al. 2002), disease (Wright et al. 2000), hyperthermia (Currie and White 1983, Flanagan et al. 1995), exercise (Walsh et al. 2001, Febbraio et al. 2002, Pösö et al. 2002) and age (Rea et al. 2001) have an effect on HSP72 expression. When the synthesis of HSP is stimulated, its accumulation is regulated from a specific DNA sequence, the heat shock element (HSE), located upstream from the coding regions of various HSP genes. HSE is a binding site for heat shock transcription factor (HSF). In unstressed cells, HSF is present in the cytoplasm and nucleus in a HSP-bound, monomeric state and has no DNA binding activity. After exposure to a stress factor, HSP binds to the damaged proteins and liberates HSF, which undergoes a conversion to a trimeric state and acquires DNA binding activity (Morimoto et al. 1994). Trimers of HSF accumulate in the nucleus and bind to the HSE and activate the gene. When sufficiently high concentrations of HSP have been synthesized, HSP combines with HSF, which thereafter cannot bind to HSE, and the synthesis of HSP decreases (Liu and Steinacker 2001).

The regulation of HSP stress response is mediated through both transcription and translation. Transcriptional response of HSP induction after heat shock seems to be proportional to the duration and the severity of the stress (Moseley et al. 1993). HSP synthesis is turned on by HSF, but synthesis is not regulated via the induction of HSF synthesis, which is done constitutively (Morimoto et al. 1994). The extent of heat shock response differs between individuals, which suggests that transcriptional regulation is related to polymorphisms in the *hsp70.2* promoter area (Schwerin et al. 2001). At least six polymorphisms have been identified in the promoter area of the *hsp70.2* gene in pigs: C/A transversion (-420 bp from the start site of translation, location 44 in Chen et al. 2000), C/A transversion near the GC box (-232 bp from the start site of translation, location 232 in Chen et al. 2000, location -47 in Schwerin et al. 1999, 2001), deletion of A near the TATA box (-214 bp from the start site of translation, location 250 in Chen et al. 2000, location -29 in Schwerin et al. 1999), G/A transversion (-191 bp from the start site of translation, location -6 in Schwerin et al. 2001) and two T/C transversions (-118 and -70 bp from the start site of translation, locations 345 and 393, respectively, in Chen et al. 2000). Because these variants found in recent pig breeds have also been found in wild pigs, these polymorphisms may be retained due to a random fixation or a consequence of breeding selection (Schwerin et al. 2001). However, frequencies of genotypes are significantly different between breeds, suggesting that promoter variants are probably not neutral in selection (Schwerin et al. 2001).

The mutations in the *hsp70.2* gene promoter area have been proposed to influence the stress-induced synthesis of HSP72 (Schwerin et al. 2001) and even meat quality (Schwerin et al. 1999). When tested in cultured fibroblasts, C/A transversion (-232) in the *hsp70.2* promoter resulted in a significantly smaller activity after heat shock than in the wild-type promoter (Schwerin et al. 2001). Schwerin et al. (1999) also reported that a C/A mutation near the GC box affects colour brightness and conductivity, and a deletion of adenosine near the TATA box affects conductivity of meat.

Translational control of HSP mRNA may be related to adenine- and uracil-rich sequence in the 3'-untranslated region that targets mRNAs for rapid turnover (Moseley et al. 1993) or for HSP72

mRNA stabilization (Gutierrez and Guerriero Jr. 1995). Schwerin et al. (2002) have also found two 3'-UTR variants in the *hsp70.2* gene of pigs that seem to affect mRNA stability.

The time scale of HSP72 expression is wide. The amount of HSP72 synthesized and how long HSP72 remains above basal levels after a stimulus are dependent on the level of stress, exposure duration, and cell type (Kiang and Tsokos 1998). An increase in HSP72 mRNA can be seen in muscles immediately after exhaustive exercise (Liu and Steinacker 2001, Pösö et al. 2002), but it takes more time to see the changes in the expression of HSP72 protein. In human muscle, the HSP72 protein content starts to rise only 24 h after exercise and continues to increase until six days post-exercise (Khassaf et al. 2001). However, in rat tissues, HSP72 protein can be detected 2.5 h after stimulus, and concentrations stay above basal levels from days to weeks afterwards (Currie and White 1983). In pigs, weaning has been shown to cause a transient increase in the expression of HSP72 in the gastrointestinal tract (David et al. 2002). Increases in the HSP72 protein content were apparent in the stomach and duodenum at 6 h after weaning, which was the time point when the first samples were taken (David et al. 2002). Thus, large inter-species variation appears to exist in HSP induction.

The protective role of HSP72 has been demonstrated in several studies. It has a protective effect after severe trauma (Pittet et al. 2002) and in patients with coronary artery disease (Zhu et al. 2003). Protection against oxidative stress has been shown to be interrelated to the function of HSPs (Papp et al. 2003). However, chronic stress seems to exhaust the chaperone-induction signalling mechanisms of HSPs, and after a large stress transient chaperone overload prevents the conformational repair of misfolded proteins (Papp et al. 2003). Lepore et al. (2001) have found a protective effect of small priming stress exposure against ischaemia-reperfusion injury in cardiac and skeletal muscle. They also noted that priming stress can induce a variety of stress-related proteins, including HSP72, which play a key role in this protection.

The HSPs are intracellular proteins because HSP72 lacks a signal peptide (Gunther and Walter 1994) needed for protein transport through the plasma membrane. Necrotic cell death and tissue injury have been shown to increase the release of HSP72 into serum (Kimura et al. 2004). Walsh et al. (2001) reported that the increase in HSP72 during physical exercise does not originate from skeletal muscle, and Febbraio et al. (2002) speculated that during physical exercise HSP72 is released from such visceral organs as the liver and intestines. HSP72 has also been found in the serum of healthy unstressed individuals, but the tissues from which HSP72 is released into the blood of healthy animals remain unknown (Romo-Figueroa et al. 1997, Pockley et al. 1998). In addition, exosomes contain HSP72 (Johnstone 2005), and exosomes and lipid rafts are proposed to be important mediators of the stress-induced release of HSP72 (Asea 2005, Lancaster and Febbraio 2005). Extracellular HSP72 is suggested to act as a danger signal to immune cells, to promote immune responses and to improve host defence (Campsi et al. 2003).

In pigs, plasma HSP72 concentrations were shown not to differ between animals with and without signs of stress (muscle and tail tremors, alternating blanched and reddened areas of skin and excitability), but whether the induction of stress proteins was only beginning at the time of sampling has not been confirmed (Romo-Figueroa et al. 1997). Van Laack et al. (1993) also did not find a relationship between stress, based on meat quality (water-holding capacity, cooking loss and colour), and HSP72 expression. In their study, the Western blot technique was used to measure HSPs, which is only a semi-quantitative method, and the number of animals was only 12, an insufficient group size to uncover any differences, especially with this type of method.

2.5 Pork quality

Meat quality is a general term for multiple interacting factors and is described as the sum of chemical, sensory, nutritional, hygienic, toxicological and technological properties of meat. Chemical quality includes the meat content of water, proteins and fat. Sensory quality includes flavour (combination of taste and odour), appearance (shape, size, colour, etc.) and texture (tenderness, juiciness, etc.). Nutritional quality consists of chemical composition and physiological value, whereas hygienic and toxicological qualities consist of microbes and residues, such as antibiotics, hormones and toxic heavy metals. Technological quality includes drip loss, amount of connective tissue and fat, meat colour and pH value (Fjelkner-Modig 1985, Hofmann 1990).

Several attributes are commonly used to describe meat quality: pH and temperature at various times after slaughter, drip loss (water-holding capacity) and colour, which are affected by almost all of the above-mentioned factors, among others. Meat quality characteristics are influenced by many intrinsic and external factors and their combinations, such as management system, breed, genotype, feeding, pre-slaughter handling, stunning, slaughter method, chilling of the carcass and storage conditions. Ethical aspects are also considered a feature of meat quality (Branscheid 1998).

Meat pH is the most important free variable in pork, and it is also strongly linked to stress. It has a direct or indirect influence on many technological characteristics of meat, including water-holding capacity (drip loss), colour, tenderness, juiciness and flavour (Kauffman et al. 1993). pH is usually measured 45 min (pH_1) and 24 h after slaughter (pH_u), the latter of which in the case of pigs is denoted ultimate pH. Ultimate pH of the meat primarily depends on the muscle glycogen content at slaughter (Bendall 1973), but also on the degree of transformation of glycogen into lactic acid and the buffering capacity of the muscle (glycolytic potential, Sellier et al. 1988). In normal situations, muscle glycogen content at the moment of slaughter is higher than 53 mmol/kg (wet weight), which is sufficient to lower the ultimate muscle *post-mortem* pH to 5.4-5.7 for good meat quality. However, during stress or after long fasting, muscle glycogen content at the time of slaughter may be insufficient, and the meat pH remains higher than 5.8, which is disadvantageous for meat quality (Henckel et al. 2002). The rate at which muscle pH falls also influences meat quality. Carcasses exhibiting a rapid pH decline have poorer meat quality than carcasses with a slow decrease in pH (Bendall et al. 1963). Carcass temperature, muscle fibre type, hormone secretion and activation intensity of muscle fibres before and during the slaughter process affect the rate of glycolysis *post-mortem* (Karlsson 1993). Degradation of muscle proteins in *post-mortem* muscle is associated with indices of meat tenderness and drip loss. Many proteins involved in tenderization and drip loss are substrates of Ca-activated proteases, calpains (Huff-Lonergan et al. 1996).

Meat colour is related to intrinsic and extrinsic factors. Age, breed, sex and muscle type are examples of intrinsic factors, whereas pre-slaughter treatment, chilling and light exposure are the examples of extrinsic factors (Renerre 1990). Temperature and pH can have an effect both intrinsically and extrinsically. If the pH of the meat is too low, the meat proteins start to denature, impacting water-holding capacity and thus also affecting reflection of light from the meat surface. This evokes the pale colour in pork. Meat colour also depends on the concentration of the meat pigments, mainly myoglobin, and on the chemical state of it (Renerre 1990). Myoglobin alternates between three oxidative stages: deoxymyoglobin (purple), oxymyoglobin (bright red) and metmyoglobin (grey-brown), the relative amounts of which determine meat colour (Hood and Riordan 1973). Even small amounts of metmyoglobin (<20%) have been found to reduce the sale of products (Hood and Riordan 1973). The colour of meat is usually measured by tricolorometric measurements, where L*, a* and b* are the colour determinants reflecting lightness, redness and yellowness, respectively.

Another important feature in meat quality is fat content and its composition, oxidative stability and uniformity, which are mainly affected by genotype and feeding strategy (Rosenvold and Andersen 2003).

2.6 Pig breeds

In Finland, the breeding of Yorkshire and Landrace pigs, the breeds used in this study, started at the beginning of the 1900s. Yorkshire pigs were brought from England, and focused breeding of Finnish Landrace began as well. The most important selection criteria in pig breeding have been daily weight gain, carcass leanness and fertility (Juga et al. 1999).

Ruusunen and Puolanne (1997) reported that variation in muscle fibre type composition within Finnish Landrace and Finnish Yorkshire is larger than the average variation within these pig breeds. LD muscle of Landrace pigs contained a higher number of slow oxidative fibres with a smaller cross-sectional area and a lower number of fast glycolytic fibres with a larger cross-sectional area than Yorkshire pigs. Based on this, the area percentage of slow oxidative and fast glycolytic fibres was about the same in both breeds. In *M. adductor*, no differences were present between the breeds in either the average cross-sectional area of the muscle fibres or the average cross-sectional area of different fibre types (Ruusunen and Puolanne 1997). The pH_1 value of Landrace pigs was lower than that of Yorkshire pigs (Ruusunen et al. 1996). In addition, Landrace pigs had a higher percentage of LDH1 isoenzyme and higher a* value than Yorkshire pigs (Suuronen 1995). In LD muscle, CS activity was higher and LDH activity lower in Swedish Landrace than in Swedish Yorkshire pigs, and also HAD activity had a tendency of being higher in Landrace than in Yorkshire pigs, but the difference was not statistically significant (Essén-Gustavsson and Fjelkner-Modig 1985). The same tendency in LDH, CS and HAD activities was also found between Finnish Landrace and Yorkshire breeds, but the differences were not significant (Suuronen 1995). Differences in meat colour have also been detected between Finnish Landrace and Yorkshire pigs. The LD muscle in Landrace breeds is lighter (L*), but redder (a*) and more yellow (b*) than in Yorkshire breeds (Kangasniemi 1993). However, in many muscle properties, high inter-individual variation exists within Landrace pigs. In Yorkshire pigs, this variation is also high but not as pronounced (Suuronen 1995).

Many of these muscle characteristics show a trend toward a higher oxidative capacity in Landrace than in Yorkshire pigs, but as discussed, inter-individual variation within a breed may also be large.

3. Aims of the study

The domestic pig is a highly inactive animal that has been bred effectively for rapid growth rate and large muscle mass over several decades, which is why the physiology and anatomy of this species is exceptional. Its skeletal muscles have been forced to adapt to the accumulation of lactate and thus to continual acidification of muscle cells. This makes it a different and very interesting animal for research. The aim in this study was to determine whether some physiological characteristics of skeletal muscle (e.g. MCT and enzyme activities) and intestines (MCT) in domestic pigs are different between individuals or from previously published data for other animals. Another goal was to specify the stress that domestic pigs experience and how to measure it reliably. For this reason, the occurrence of HSP72 in porcine tissues was measured, as were some conventional stress markers (growth, blood lactate, and serum concentrations of cortisol, glucose, free fatty acids and haptoglobin).

Specific aims of this research were to investigate the following:

1) the distribution and expression of MCT isoforms in muscles of the domestic pig and wild boars and to compare these data with meat quality (Study 1 and unpublished data).

2) the expression pattern of MCT isoforms and their chaperone protein CD147 in the small intestine and the colon of Finnish Landrace and Finnish Yorkshire pigs and their crosses (Study 2).

3) whether the amount of HSP72 in the small intestine, colon or serum could be used as an indicator of stress (Study 3).

4) the promoter region of the *hsp70.2* gene in domestic pigs to determine whether known promoter polymorphisms are present in Finnish Landrace or Finnish Yorkshire breeds and wild pigs, and if so, whether they affect HSP72 expression or meat quality parameters (Study 4).

4. Materials and methods

4.1 Animals and diets

Forty pigs (20 castrated males and 20 females) from 20 litters (two per litter) were used in Studies 2, 3 and 4 (experimental pigs, EPs). The breeds used were Finnish Landrace (n=16), Finnish Yorkshire (n=16) and Landrace x Yorkshire (n=8). They were raised individually in 1.0 x 2.5 m pens at Agrifood Research Finland (Animal Production, Pigs, Hyvinkää, Finland) until they were 163–166 days old. At that time, their average live weight was 105 ± 9 kg. The EPs were weighed biweekly, and also carcass weight was recorded. On the day of slaughter, pigs were transported, two at a time, from Hyvinkää to Helsinki (approximately 1 h), and were allowed to rest 2 h before exsanguination. Pigs were slaughtered at an experimental slaughterhouse (University of Helsinki, Department of Food Technology) after electrical stunning (80 V / 10 s.). EPs were fed a high-lysine or a low-lysine diet (Ruusunen et al. 2007), but because no differences were found between groups in the amounts of HSP72 or MCTs, the groups are considered as a single group.

In Study 1, 18 pigs from the EP group (EP^{I}: 8 Finnish Landrace, 6 Finnish Yorkshire and 4 Landrace x Yorkshire) were used.

In Study 3, the EPs and 10 additional Finnish Landrace x Yorkshire pigs, which were about six months old, were used (slaughterhouse pigs 1, SP1). Five of the additional pigs were physically injured (tail biting and/or joint inflammations, which slightly impeded movement) and five had no signs of injury. Animals were transported approximately 5 km to the slaughterhouse 3 h before slaughter. Study 2 also used EPs and 8 additional slaughterhouse pigs (SP2; 4 Finnish Landrace and 4 Finnish Yorkshire; sexes randomly selected, 7 females and 1 castrated male) and Study 4, EPs and 78 additional pigs (SP3; 56 Finnish Landrace and 22 Finnish Yorkshire; 34 females, 19 intact males and 25 castrated males). From SP1 and SP2, carcass weight was measured. All of the SP samples were taken at a commercial slaughterhouse (Koiviston teurastamo, Mellilä, Finland). These samples were taken at slaughter (SP1 and SP2) or 24 h after slaughter (SP3). In the slaughterhouse, the pigs were stunned with CO_2 (Barton Gade et al. 1995).

Samples were also taken from 6 wild pigs, aged 1-3 years, to analyse muscle MCT distribution and *hsp70.2* promoter polymorphisms. The wild pigs were raised in an enclosed area in the forest, they were killed by shooting, exsanguated and then transported for approximately 1.5 h to the slaughterhouse.

Table 1 presents an overview of the experimental design in Studies 1-4 and experiments performed on wild pigs.

Table 1. Overview of experimental design in Studies 1-4 and in studies on wild pigs (unpublished data).

Study	Code	No. of animals	Breeds	Analyses	Tissue
I	EP[I]	18	8 Landrace 6 Yorkshire 4 crosses	MCT, lactate, LDH, CS, HAD	skeletal muscle, blood, serum
II	EP	40	16 Landrace 16 Yorkshire 8 crosses	MCT	small intestine, proximal colon
	SP2	8	4 Landrace 4 Yorkshire	MCT	small intestine (1 m and 12 m from pylorus)
III	EP	40	16 Landrace 16 Yorkshire 8 crosses	HSP72 protein, lactate, cortisol, FFA, glucose, haptoglobin	small intestine, colon, blood, serum
	SP1	10	10 crosses	HSP72 protein, lactate, cortisol, FFA, glucose	small intestine, colon, blood, serum
IV	EP	40	16 Landrace 16 Yorkshire 8 crosses	*hsp70.2* promoter analysis, HSP72 protein, meat quality	skeletal muscle (*longissimus*)
	SP3	78	56 Landrace 22 Yorkshire	*hsp70.2* promoter analysis, HSP72 protein, meat quality	skeletal muscle (*longissimus*)
-	Wild pigs	6		MCT, lactate, LDH, CS, HAD	skeletal muscle, intestines, blood, serum

4.2 Samples

Muscle samples were taken from EPs as soon as possible after stunning from five different muscles: LD (15-18 min), M (15-18 min), IS (15-18 min), G (25-30 min) and S (approximately 40 min). The numbers in parentheses indicate the sampling times. All samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis. The muscle samples from wild pigs and SP1 (IS) were taken in the same manner, but the delay after stunning was longer, about 1.5 h. The muscle samples from SP3 (*M. semispinalis capitis*, LD and S) were taken 24 h after slaughter and stored at -20°C until analysis. pH values were measured in muscles 45 min and 24 h after stunning before freezing.

The samples from the intestines (EPs and SP1) were taken approximately 50 min after stunning. The samples were washed, blotted dry, frozen in liquid nitrogen and stored at -80°C until analysis. The sample of the small intestine was taken about 2.5 m from the pylorus, and the colon sample from the proximal colon. The small intestinal SP2 samples were treated as described above, but the samples were taken at 1 m and 12 m from the pylorus.

In addition, liver samples from EPs were obtained about 30 min after stunning and treated similar to muscle samples. Blood samples (EPs, SP1 and wild pigs) were taken from each pig at bleeding. Blood samples were divided into EDTA, plain and Na-fluoride-containing tubes to obtain plasma, serum and whole blood, respectively. Samples were stored on ice until plasma/serum was separated.

4.3 Methods

4.3.1 Biochemical analyses

For lactate and glycogen determinations, muscle samples were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.0). Muscle lactate concentrations were analysed (EPs, wild pigs) spectrophotometrically using a commercial kit (no. 139 084, Boehringer-Mannheim, Mannheim, Germany), and blood lactate concentration was measured (EPs, SP1, wild pigs) with a lactate analyser (YSI 2300 STAT Plus, YSI Inc., Ohio, USA). Glycogen concentration in muscle (EPs; Study 1) was determined by hydrolysing the homogenate in 0.1 M HCl at 100°C for 2 h and thereafter adjusting the pH to 6.5–7.5 (Lowry and Passoneau 1973). For muscle glucose measurements, a Roche diagnostic kit (no. 1447521) was used. The glycolytic potential (GP) was calculated according to Monin and Sellier (1985): GP = 2([glycogen] + [glucose] + [glucose-6-phosphate]) + [lactate]. Total activity of LDH (EPs, wild pigs) was determined according to the recommendation of the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (1974), and LDH isoenzymes were separated by electrophoresis (Paragon®, Beckman, Fullerton, USA). The isoenzyme composition was used to calculate the percentage of H (heart) and M (muscle) chains of the total LDH activity. HAD (EPs, wild pigs) was measured with the method of Gottesmann and Hamm (1986), and CS (EPs, wild pigs) according to Srere (1969).

Serum cortisol (EPs, SP1; Study 3) was measured by radioimmunoassay (Coat-A-Count® Cortisol kit, DPC, Los Angeles, USA), and free fatty acids and glucose (EPs, SP1) by spectrophotometry (Trinder 1969, Shimizu et al. 1980). The haptoglobin method (EP) used was based on detection of peroxidative activity of the haptoglobin-haemoglobin complex (PHASE RANGE Haptoglobin assay, Tridelta Development Ltd., Wicklow, Ireland).

4.3.2 Meat quality analysis

The meat quality analyses are described in detail in Study 4. The pH values were measured by an Xerolyt electrode (Mettler-Toledo Inlab 427) from meat extracts using a Knick Portamess 752 pH-

meter. From muscles, taken 45 min (EPs) post-mortem (pH_1), homogenate in iodoacetate solution was made, and the pH of this homogenate was measured. The pH 24 h (pH_u) post-mortem was measured straight from the muscle (EPs, SP3). Drip loss measurements (EPs) were done according to Honikel (1985), and meat colour (L*, a* and b*) was assessed with a Minolta CR-200 (EPs) or a Minolta CR-300 (SP3) device (Minolta Camera Co., Japan) from three random spots.

4.3.3 Determination of MCT isoforms

MCT isoforms and CD147 were analysed by immunoblotting as described in Study 1. For MCT2 (Chemicon International, Billerica, USA) and CD147 (R&D Systems, Minneapolis, USA), commercial primary antibodies were used. MCT1 and MCT4 antibodies were made in rabbits by Sigma Genosys (Cambridge, UK). The secondary antibody used for MCT1 and MCT4 was horseradish peroxidase (HRP) conjugated goat anti-rabbit immunoglobulin (DAKO, Glostrup, Denmark). For MCT2, HRP-conjugated anti-chicken secondary antibody (Chemicon International) was used and for CD147, HRP-conjugated rabbit anti-goat immunoglobulin (DAKO).

Membrane samples, prepared by the Percoll method (Jackson et al. 1997), were separated on SDS-PAGE gels and electrotransferred to a nitrocellulose membrane (Protran®, Schleicher & Schuell, Dassel, Germany). Membranes were incubated with the respective primary and secondary antibodies, and the bands were detected using a chemiluminescent method (SuperSignal® West Dura Extended Duration Substrate; Pierce, Rockford, USA). Membrane samples from pig heart and small intestine were used as positive controls for MCT1, and membranes from rat liver served as a positive control for MCT2. The rat liver sample was a generous gift from the National Health Institute (Helsinki, Finland).

4.3.4 Determination of HSPs

HSP72 was analysed from the small intestine (EPs, SP1), colon (EPs, SP1), serum (EPs, SP1) and IS (SP1) and LD (SP3) muscles by an Hsp70 EIA kit (StressGen Biotechnologies Corp., Victoria, Canada). The producer of the kit has confirmed that the antibody recognizes porcine HSP72. Tissues were homogenized with FastPrep™ homogenizer (FP120, Bio101, ThermoSavant; Savant Instruments, Holbrook, USA). The method is described in more detail in Study 3.

4.3.5 DNA extraction, PCR analysis of hsp70.2 and sequencing of PCR products

DNA from the domestic pig (EPs, SP3) and wild pig tissues was separated by the method of Miller et al. (1988). Polymerase chain reaction (PCR), described in Study 4, was used to amplify the *hsp70.2* promoter region for sequencing. The sequencing was performed at a DNA sequencing laboratory (Institute of Biotechnology, University of Helsinki, Finland).

4.3.6 Statistical analysis

Results are shown as means ± standard deviation (SD). Differences between muscles (Study 1) and breeds (all studies) were tested with repeated measures analysis of variance, and when the overall differences were significant, the individual muscles (domestic pigs and wild pigs) or breeds (domestic pigs) were compared with Tukey's post-test. In Study 3, differences in the amounts between the small intestine and colon were calculated by Student's paired t-test. Differences between different *hsp70.2* alleles (Study 4) were calculated by Student's t-test or with one-way analysis of variance with Tukey's post-test. In Study 2, analyses were performed using analysis of variance (GenStat Version 8), followed by LSD tests. These analyses are described in detail in Study 2.

Linear relationships between variables were examined using correlation analysis (Pearson's correlation coefficients). GraphPad Prism, version 3.03 for Windows (GraphPad Software,

www.graphpad.com) and SAS System for Windows, V8.2 (SAS Institute, 2000) were used for statistical calculations. Differences were regarded as significant at $P<0.05$.

5. Results

5.1 Carcass weight

The mean carcass weight of the 40 EPs was 81.0 ± 7.5 kg. Among the EPs, no significant difference was present in carcass weight between Yorkshire and Landrace pigs, but crosses were heavier ($P<0.05$) than pure-breeds. The total growth rate of EPs (birth to slaughter) was 627 ± 51 g/day and final live weight 105 ± 9 kg. In EPs, growth rate and final live weight correlated with carcass weight (both $r = 0.96$, $P<0.001$). Carcass weight of SP1, 74.7 ± 10.9 kg, was significantly ($P<0.05$) lower than that of EPs. The carcass weight of SP2 was 76.2 ± 3.6 kg. No carcass weight data are available for SP3. The carcass weights of the wild pigs were 72.7, 79.9, 29.2, 46.6, 32.4 and 43.7 kg. The average lean meat content of domestic pigs was 48.8 ± 4.7 kg and of wild pigs 25.9 ± 9.9 kg. Live weight of wild pigs was not recorded.

5.2 Lactate concentrations

The mean lactate concentrations in muscle samples of EP^I, taken in association with slaughter and frozen immediately in liquid nitrogen, were as follows: LD 56 ± 14 mmol/kg, S 59 ± 15 mmol/kg, G 52 ± 14 mmol/kg, M 30 ± 5 mmol/kg and IS 40 ± 9 mmol/kg wet weight (w.w.). The mean blood lactate concentration in EPs was 2.9 ± 1.7 mmol/l and in SP1 5.5 ± 1.9 mmol/l, which is higher ($P<0.001$) than the concentration in the EP group. The muscle lactate concentration was not determined from SP1, and muscle or blood lactate was not determined from SP2 or SP3.

The mean lactate concentrations in muscles of wild pigs (all expressed as mmol/kg wet weight) were the following: LD 72 ± 19, S 64 ± 14, G 64 ± 10, M 27 ± 5 and IS 39 ± 7. Blood lactate concentrations in wild pigs were 2.9, 1.6, 10.2, 2.7, 1.1 and 1.4 mmol/l. Lactate concentration in the G muscle of wild pigs was higher than in domestic pigs ($P<0.05$), but in other muscles differences were not significant ($P>0.05$).

5.3 3-hydroxyacyl-CoA dehydrogenase, citrate synthase and lactate dehydrogenase

In 18 EP (EP^I), activities of 3-hydroxyacyl-CoA dehydrogenase (HAD) were 4.8 ± 0.8 U/g in LD, 6.8 ± 1.1 U/g in S, 6.6 ± 0.9 U/g in G, 10.1 ± 2.3 U/g in M and 12.6 ± 2.4 U/g in IS. The activities of citrate synthase (CS) were 3.9 ± 0.8 U/g, 5.9 ± 1.1 U/g, 5.9 ± 1.2 U/g, 9.2 ± 1.9 U/g and 9.3 ± 1.8 U/g, respectively (Table 2). Activity of HAD, which is a marker of the capacity of β-oxidation of fatty acids, was significantly lower in the LD muscle than in M ($P<0.001$), IS ($P<0.001$), S ($P<0.01$) and G ($P<0.01$) muscles. The highest activity was measured in IS ($P<0.001$ for all other muscles), but also in the M muscle the activity of HAD was significantly higher ($P<0.001$) than in the glycolytic LD, G and S muscles. CS is a marker of the muscle's oxidative capacity, and it showed similar distribution as HAD. The lowest activity was in LD, and the difference in activity between the oxidative (M and IS) and glycolytic (LD, G and S) muscles was significant ($P<0.001$). No difference was found between the oxidative muscles IS and M.

Table 2. Mean activities (U/g, wet weight) of lactate dehydrogenase (LDH), 3-hydroxyacyl-CoA dehydrogenase (HAD) and citrate synthase (CS) as well as the percentage of LDH H-chain in five muscles of wild pigs (WP, n=6) and domestic pigs (EP^I, n=18). In wild pigs, *M. longissimus dorsi* differed significantly from the other muscles for HAD and CS. These differences are shown as *** (P<0.001), ** (P<0.01) and * (P<0.05). In EP^I, the values indicated with the superscript A differed significantly (P<0.001) from values indicated with the superscript B. Other differences are given in the text.

Muscle	**LDH**		**LDH H-chain (%)**		**HAD**		**CS**	
	WP	**EP^I**	**WP**	**EP^I**	**WP**	**EP^I**	**WP**	**EP^I**
M. longissimus dorsi	447 ± 157	679 ± 360^{A}	2.7 ± 3.5	4.9 ± 5.0^{A}	11.0 ± 1.3	4.8 ± 0.8^{A}	9.4 ± 1.6	3.9 ± 0.8^{A}
M. masseter	205 ± 99	146 ± 75^{B}	52.0 ± 17.7	30.3 ± 12.6^{B}	16.5 ± 2.5**	10.1 ± 2.3^{B}	13.6 ± 2.6**	9.2 ± 1.9^{B}
M. gluteus superficialis	383 ± 163	668 ± 269^{A}	19.1 ± 13.7	7.1 ± 5.3^{A}	15.7 ± 4.0*	6.6 ± 0.9^{A}	15.1 ± 4.2*	5.9 ± 1.2^{A}
M. infraspinatus	252 ± 121	278 ± 118^{B}	29.7 ± 5.8	27.9 ± 12.3^{B}	18.1 ± 2.7***	12.6 ± 2.4^{B}	13.7 ± 2.1**	9.3 ± 1.8^{B}
M. semimembranosus	640 ± 342	745 ± 413^{A}	3.7 ± 4.6	7.6 ± 8.0^{A}	15.8 ± 1.6***	6.8 ± 1.1^{A}	15.2 ± 2.8**	5.9 ± 1.1^{A}

In Study 1, total LDH activity and the proportions of its five isoenzymes in the muscles were measured from EP[I] (Study 1; Figure 1, Table 1). The LDH activity was the lowest in the M (146 ± 75 U/g) and IS (278 ± 118 U/g) muscles and significantly higher in glycolytic LD (679 ± 360 U/g), G (668 ± 269 U/g) and S (745 ± 413 U/g) muscles ($P<0.001$). LDH5 was the dominant isoenzyme in all muscles, whereas the activity of LDH1 was minor. The percentage of H-chains was calculated, and it showed an opposite distribution to total LDH, i.e. the highest percentages were found in M and IS and the lowest in LD, G and S muscles. When all samples from the 18 pigs were combined, a negative correlation was found between total LDH activity and the number of H-chains ($r = -0.98$, $P<0.001$).

The LDH, CS and HAD activities of wild pigs are also shown in Table 2, and the distributions of LDH isoenzymes in different muscles in Figure 1. CS activity was significantly higher ($P<0.01$) in all wild pig muscles than in the respective muscles of domestic pigs, which was also true for HAD activity in all muscles but S. The activities of HAD and CS in the muscles of wild pigs were lower in LD than in the other muscles examined (Table 2).

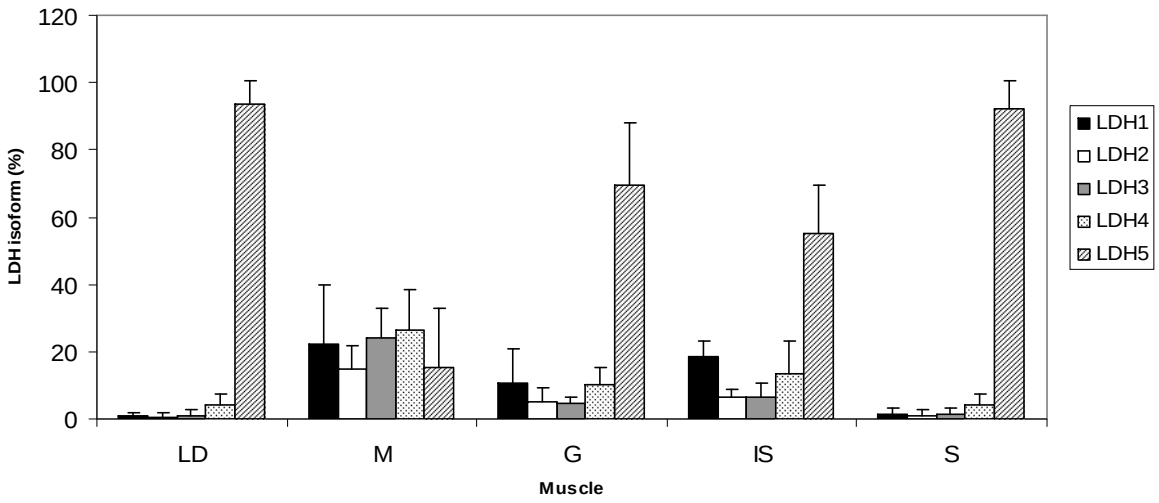

Figure 1. Distribution of lactate dehydrogenase (LDH) isoenzymes in *M. longissimus* (LD), *M. masseter* (M), *M. gluteus* (G), *M. infraspinatus* (IS), and *M. semimembranosus* (S) wild pigs.

No significant differences were present between the other muscles. The total activity of LDH did not differ significantly from the respective muscle in EP in any of the muscles evaluated. The number of H-chains (%) was higher in M ($P<0.05$) and G ($P<0.05$) of wild pigs than of EPs. Total LDH activity was higher in LD and S than in IS or M ($P<0.05$). LDH activity was also higher in G than in M ($P<0.05$), but not in IS ($P=0.09$). No difference was observed in LDH activity between LD, S and G or between IS and M. The number of H-chains (%) was higher in M than in other muscles (LD, $P<0.001$; S, $P<0.001$; G, $P<0.01$ and IS, $P<0.05$) and lowest in LD and S (G, $P<0.05$ and IS, $P<0.001$). No difference in H-chain percentage existed between G and IS.

The HAD, CS or LDH activities were not measured in SPs.

5.4 Free fatty acids, cortisol, glucose and haptoglobin

The average concentrations of FFA, glucose and haptoglobin are shown in Study 3, Table 1. In the EP group, blood and serum concentrations of metabolites showed no significant differences between breeds. Differences were found between EPs and SP1 in concentrations of FFA and glucose ($P<0.001$), with concentration of glucose being higher in SP1, and FFA higher in EPs. A negative correlation ($r = -0.61$, $P<0.001$, $n = 50$) was present between glucose and FFA concentrations. Cortisol and FFA ($r = 0.37$, $P<0.01$, $n = 50$) correlated in the whole material (Study 3) and also in the two groups, EP and SP1, distinctly ($r = 0.42$, $P<0.01$, $n = 40$; $r = 0.65$, $P<0.05$, $n = 10$). Cortisol concentration showed a positive correlation with carcass weight ($r = 0.28$, $P<0.05$, $n = 50$). Haptoglobin showed no correlation with any of these parameters or with carcass weight among EPs.

5.5 Meat pH and colour

Meat colour was analysed in EPs and SP3 (Table 3). Data from these groups were analysed separately because meat quality tests for these groups were done in two separate laboratories, and could not be compared with each other. Sex affected only lightness (L* value) in SP3, with the S muscle of female pigs being darker (lower L* value) than that of castrated males ($P<0.05$), but not significantly different from the value in intact males.

Moreover, breed had some effects on meat quality. In SP3, redness (a* value, $P<0.001$) and yellowness (b* value, $P<0.01$) of the neck (*M. semispinalis capitis*) were greater in Landrace than Yorkshire pigs. In S muscle, the pH_u ($P<0.05$) and b* value ($P<0.01$) were higher in Yorkshire than in Landrace pigs (SP3).

Table 3. Meat quality parameters of 118 Finnish Landrace and Finnish Yorkshire pigs and their crosses (EP and SP3).

	EP			**SP3**	
	Landrace (n=16)	Yorkshire (n=16)	Crosses (n=8)	Landrace (n=56)	Yorkshire (n=22)
L*					
Semimembranosus	58.9 ± 2.2	58.6 ± 3.1	60.4 ± 3.0	60.9 ± 3.2	61.7 ± 2.6
Longissimus dorsi	56.9 ± 2.2	56.2 ± 4.2	57.8 ± 2.8	55.1 ± 2.5	54.6 ± 2.8
Semispinalis capitis	ND	ND	ND	56.2 ± 3.3	57.8 ± 3.6
a*					
Semimembranosus	5.5 ± 0.9	5.5 ± 1.2	5.6 ± 1.1	5.4 ± 1.8	5.6 ± 1.2
Longissimus dorsi	5.8 ± 0.9	5.4 ± 1.4	6.2 ± 1.5	6.8 ± 1.1	6.8 ± 0.9
Semispinalis capitis	ND	ND	ND	11.7 ± 2.7	8.9 ± 1.4***
b*					
Semimembranosus	2.8 ± 0.7	2.7 ± 0.9	3.3 ± 0.7	4.1 ± 1.4	4.9 ± 1.1**
Longissimus dorsi	2.0 ± 1.1	1.4 ± 0.7	1.9 ± 1.1	3.8 ± 0.9	3.9 ± 0.7
Semispinalis capitis	ND	ND	ND	6.7 ± 1.3	5.8 ± 1.4**
pH_u					
Semimembranosus	5.4 ± 0.0	5.5 ± 0.1	5.4 ± 0.0	5.7 ± 0.2	5.8 ± 0.2*
Longissimus dorsi	5.4 ± 0.1	5.5 ± 0.1	5.4 ± 0.0	5.5 ± 0.1	5.5 ± 0.1
Semispinalis capitis	ND	ND	ND	5.8 ± 0.3	5.7 ± 0.2
Drip loss					
Semimembranosus	9.2 ± 2.7	7.5 ± 2.6	8.3 ± 2.2	ND	ND
Longissimus dorsi	12.3 ± 3.1	10.0 ± 3.7	11.6 ± 2.5	ND	ND
pH_1					
Longissimus dorsi	6.1 ± 0.4	6.3 ± 0.3	6.2 ± 0.3	ND	ND

* Significantly different from Landrace pigs, P<0.05 (Group B)
** Significantly different from Landrace pigs, P<0.01 (Group B)
*** Significantly different from Landrace pigs, P<0.001 (Group B)
ND = not determined

5.6 Monocarboxylate transporters and CD147

5.6.1 Skeletal muscle

MCT1, MCT2 and MCT4 were detected in the muscles of both domestic (Study 1, Figure 2) and wild pigs. The apparent size of MCT1 was about 55 kDa, MCT2 40 kDa, and MCT4 50 kDa.

In rat liver membranes used as a control, the molecular weight of MCT2 was about 48 kDa. In all porcine muscles studied, the content of MCT1 was too small and variable to be accurately detected, and therefore, no comparisons between muscles or between domestic and wild pigs were done. In membrane samples from the porcine heart and small intestine, the anti-MCT1 antibody gave a strong signal, indicating that the anti-MCT1 antibody used in these studies recognizes porcine MCT1.

In domestic pigs, the content of MCT4 was similar in oxidative and glycolytic muscles (Table 4), and no differences were found between Landrace and Yorkshire pigs. The amounts of MCT4 were significantly higher in almost all muscles of wild pigs than in domestic pigs and more clearly so in anaerobic LD ($P=0.01$), S ($P<0.001$) and G muscles ($P<0.001$) than in more aerobic IS ($P=0.08$) and M ($P<0.05$) muscles. Figure 2 shows the Western blot results of MCT4 analysis in the LD muscle of wild pigs compared with domestic pigs.

The mean amount of MCT2 in the domestic pig was higher ($P<0.01$) in glycolytic LD, G and S muscles than in the more oxidative M and IS muscles (Table 4). No breed difference in the amounts of MCT2 was found. In wild pigs, the amount of MCT2 did not differ significantly from that in domestic pigs.

Table 4. Relative amounts (arbitrary optical density, OD) of isoforms MCT2 and MCT4 in experimental pigs (EP^I, n = 18) and wild pigs (WP, n = 6). The values marked with A differed significantly ($P<0.05$) from the values marked with B.

	EP^I		WP	
Muscle	MCT2	MCT4	MCT2	MCT4
G	169 ± 69 ^{A}	155 ± 66	137 ± 56	325 ± 32
IS	100 ± 40 ^{B}	184 ± 71	125 ± 35	239 ± 69
LD	147 ± 45 ^{A}	156 ± 60	186 ± 61	257 ± 68
M	105 ± 50 ^{B}	168 ± 57	131 ± 55	240 ± 71
S	147 ± 68 ^{A}	162 ± 65	150 ± 64	267 ± 22

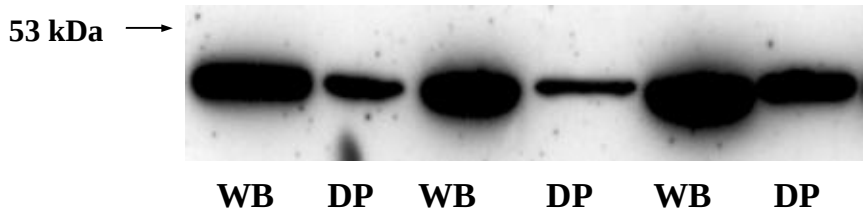

Figure 2. Western blot of MCT4 in the *M. longissimus dorsi* of wild pigs (WP) and domestic pigs (DP). The nearest molecular weight standard is shown on the left.

In all muscles, excluding IS, a negative correlation was found between the contents of MCT2 and MCT4 (Study 1, Figure 3). The correlations were r = -0.66 (P<0.01), -0.58 (P<0.05), -0.63 (P<0.01) and -0.65 (P<0.01) in M, LD, S and G, respectively. Glycolytic potential was calculated from concentrations of glycogen and lactate in muscle (results not shown). In the five muscles investigated, a significant correlation (r = 0.91, P<0.05, n = 5) was present between the average values of glycolytic potential and MCT2, while the correlation between the content of MCT4 and glycolytic potential was not significant. When results from all muscles were combined, negative correlations between the content of MCT2 and the activity of CS (r = -0.27, P<0.01) as well as between the content of MCT2 and the activity of HAD (r = -0.32, P<0.01) were observed, but the corresponding correlations between MCT4 and HAD or CS were not significant. A negative correlation (r = -0.53, P<0.05) was also present between total LDH activity and content of MCT4 in M muscles (Study 1, Figure 4), but not in other muscles.

No correlation between the amounts of MCTs and any of the meat quality parameters used (pH, drip loss, colour) was found in EPs.

5.6.2 Gastrointestinal tract

MCT1 and MCT4 were detected in both the small intestine and the colon, with the same apparent molecular weights as in skeletal muscle. MCT2 was expressed only in the small intestine at 80 kDa (Study 2, Figure 1). The detected molecular weight of CD147, a chaperone protein for MCT1 and MCT4, was 110 kDa (Study 2, Figure 1), but when some of the samples were reanalysed after approximately 1.5 years of storage of membranes at –80 C, an additional 55 kDa band appeared.

The expressions of MCT isoforms and CD147 are shown in Table 1 of Study 2. The amount of MCT4 was higher (P<0.05) in the small intestine than in the colon (Study 2, Table 1), and the amount of MCT1 in the small intestine was higher (P<0.05) in samples taken 1 m from the pylorus than in samples taken 12 m from the pylorus (Study 2; Figure 2A, Table 4).

The amounts of MCTs were similar in the two breeds, but the amount of CD147 was smaller in the small intestine of Landrace than Yorkshire pigs (P<0.05).

In the small intestine, a positive correlation existed between the amounts of MCT1 and MCT4 (r = 0.35, P<0.05), while no such correlation was found in the colon (P=0.07). In the small intestine, the amounts of MCT1 and CD147 also showed a positive correlation (r = 0.36, P<0.05). The amount of MCT1 in the small intestine correlated with that in the colon (r = 0.69, P<0.001), and a similar correlation was found between the amounts of CD147 (r = 0.67, P<0.001).

5.7 Heat shock protein 72

5.7.1 Concentration of HSP72

HSP72 was detected in the small intestine, colon and serum of EPs and SP1. The results are shown in Table 2 of Study 3.

The amounts of HSP in all tissues examined were higher in SP1 than EPs ($P<0.001$), and injured SP1 had higher colon HSP72 concentrations (9.0 ± 1.8 µg/g of tissue) than uninjured SP1 pigs (5.4 ± 2.0 µg/g of tissue, $P<0.05$). The amount of HSP72 was lower in the colon (3.8 ± 1.8 µg/g of tissue) than in the small intestine (9.7 ± 2.7 µg/g of tissue) ($P<0.001$, $n = 50$), a finding that was also apparent when EPs and SP1 were analysed separately ($P<0.001$; $n = 40$ for EP, $n = 10$ for SP1). Among EPs, no significant differences were found between breeds in HSP72 content. Positive correlations existed between the amounts of HSP72 in the colon and small intestine ($r = 0.54$, $P<0.001$, $n = 50$), the colon and serum HSP72 ($r = 0.66$, $P<0.001$, $n = 50$) (Study 3, Figure 1) and the small intestine and serum HSP72 ($r = 0.41$, $P<0.01$, $n = 50$). In addition, HSP72 was measured in the liver of EPs; the average concentration was 5.8 ± 1.6 µg/g of tissue. No correlations of HSP72 existed between the liver and other tissues.

HSP72 content in the colon ($r = -0.54$, $P<0.001$, $n = 50$) (Study 3, Figure 2) and serum ($r = -0.45$, $P<0.01$, $n = 50$) correlated negatively with carcass weight, but no such correlation was found with HSP72 in the small intestine. Colon HSP72 correlated with carcass weight also when the groups were analysed separately (SP1, $r = -0.70$, $P<0.05$, $n = 10$; EP, $r = -0.35$, $P<0.05$, $n = 40$). Serum HSP72 correlated with blood lactate ($r = 0.46$, $P<0.01$, $n = 50$), glucose ($r = 0.73$, $P<0.001$, $n = 50$) and FFA ($r = -0.46$, $P<0.001$, $n = 50$). No correlations between serum cortisol or haptoglobin and serum HSP72 concentrations were found.

In SP1 and SP3, the concentrations of muscle HSP72 were measured. In SP1, the concentration of HSP72 in IS was 3.9 ± 1.0 µg/g of tissue, and in SP3 the concentration of HSP72 in LD was 0.04 ± 0.05 µg/g of tissue. This large difference is most probably a consequence of different handling of the samples; SP3 samples were not frozen in liquid nitrogen, unlike SP1 samples, and SP3 samples were stored at -20°C instead of -80°C.

5.7.2 Polymorphisms in the hsp70.2 gene promoter area

In Study 4, the *hsp70.2* gene promoter area was determined in a total of 118 pigs. Two separate groups were used: one group with 40 pigs (EPs) and the other group with 78 pigs (SP3). Breeds used in this study were Finnish Landrace ($n = 72$) and Finnish Yorkshire ($n = 38$) and their crosses ($n = 8$). The genotype frequencies of seven polymorphic sites in the *hsp70.2* gene promoter area are shown in Table 1 of Study 4. The locations of the polymorphic sites are counted upstream from the translation start site and are shown in parentheses. We found C/A transversion (-232), A/G transition (-220), A/- deletion (-214), G/A transition (-191), T/C transition (-118), C/T transition (-70) and a new polymorphic site in Finnish Landrace pigs: an insertion of a 9-nucleotide sequence (GGAGAGATC) at -11. Only two polymorphisms (sites -232 and -118) were present in both breeds. Deletion of adenosine at -214 was present only in the Landrace breed and transitions at -220, -191 and -70 only in the Yorkshires. Only six wild pigs were analysed, but C/A transversion (-232) and T/C transition (-118) were found also in them.

Different alleles within polymorphisms in the promoter area were compared with meat quality data to define differences between alleles. Because some of the polymorphisms exist in only one breed, the data is analysed also within the breed to eliminate breed differences in results. Furthermore the

different groups (EP and SP3) were analysed separately, because the analysis of meat quality was performed in different laboratories. In the LD of SP3 (n = 78), a* values were higher in animals with A/A at -232 than in animals with C/C ($P<0.05$). For the polymorphic site at -118 (T/C), animals with a C/C allele had a higher a* value in LD than animals with a T/T allele ($P<0.05$). The above-mentioned differences were also found in Landrace pigs ($P<0.05$) when the data were analysed within breed, but not in Yorkshire pigs. The deletion of A (site -214) in front of the TATA box was found only in Landrace pigs. In these pigs, the L* value in *M. semispinalis capitis* was higher in animals without deletion of A (A/A) than in animals with deletion of adenosine in the other allele (A/-) ($P<0.01$). In S and LD muscles, the same tendency was observed ($P<0.10$). In S muscle of Landrace pigs, pH_u was lower in A/A than in A/- animals ($P<0.05$). In the case of G/A polymorphism at position -191, which was detected only in Yorkshire pigs, b* value in the S muscle was higher in G/G than in G/A animals ($P<0.05$). In Yorkshire pigs, pH_u in LD was lower in animals with C/T transition at -70 than in C/C animals ($P<0.05$). In EPs, drip loss was greater in pigs with an insert (at position -11) than in pigs without the insert ($P<0.05$). Due to the small number of animals with homozygous insertion, heterozygous and homozygous animals were regarded as one group (t-test).

The concentration of HSP72 protein in LD (SP3) was measured to determine whether a correlation exists between different promoter area polymorphisms and the protein concentration of HSP72. The average HSP72 content was 41 ± 55 ng/g of tissue. Yorkshire pigs (61 ± 76 ng/g) had a tendency to have a higher amount of HSP72 protein in their LD than Landrace pigs (33 ± 43 ng/g), but the difference was not significant ($P=0.06$). Pigs with a T/C transition (-118) had higher HSP72 content than animals with a T/T transition ($P<0.05$). However, the increase of HSP72 in the case of C/C pigs compared with T/T pigs was not significant ($P=0.10$) due to the very small number of C/C animals (n = 7). No other polymorphisms had a significant effect on HSP72 expression, but pigs with a C/A transversion (-232) tended to have more HSP72 in their LD than 'wild-type' C/C ($P=0.08$) pigs, and within the Landrace breed, pigs with an insert at -11 tended to have higher HSP72 contents than animals without the insert ($P=0.08$).

None of these polymorphisms significantly affected HSP72 expression in the gastrointestinal tract or HSP72 concentration in serum.

6. Discussion

6.1 Methodological considerations

The Western blot analysis used for MCT determination is a semi-quantitative method that further depends on the specificity of the antibody used. The latter is especially important in this study because none of the antibodies used was specific for porcine MCTs or CD147. The method does, however, allow comparisons of the amounts of a single MCT isoform between individuals. For the same reason mentioned above, the amounts of different MCT isoforms cannot be compared with each other. Thus, the result indicating that MCT1 was not found in some muscles with Western blot does not necessarily mean that MCT1 was not expressed in muscles, but possibly the antibody was not specific enough to show any signal.

Proper antibodies are crucial for Western blot analysis. The sequences for porcine MCT proteins were not known at the time of analysis, and thus, we could not make/order a specific antibody for porcine MCTs. Several antibodies made for other species were therefore tested to find one that gave a band at the right molecular weight, a minimum number of bands at other molecular weights and

the strongest signal. Anti-human (MCT1 and MCT4) and anti-rat (MCT2) antibodies were used, and whether these antibodies cross-react with the same MCT isoforms also in the pig is a valid question. Membranes from porcine heart and small intestine gave a strong signal with anti-MCT1 antibody, for which the size was the same as found in muscle samples. The apparent molecular weight of MCT1 found was 55 kDa, which is somewhat higher than in an earlier study of porcine colon (Ritzhaupt et al. 1998b), but consistent with predictions in other studies (Jackson et al. 1995, Koho et al. 2005). These data suggest that the antibody used did recognize MCT1 protein. Anti-MCT2 resulted in a single band, both in skeletal muscle and in the small intestine. In skeletal muscle, MCT2 had a molecular weight (40 kDa) somewhat lower than that in the rat liver membranes used as a positive control. In the small intestine, the molecular weight was 80 kDa, which is twice the value for a monomer (Garcia et al. 1995) and may, as suggested earlier, be due to dimerization (Garcia et al. 1995, Bergersen et al. 2001) or to an association with chaperone protein, such as gp70 (Wilson et al. 2005). Variation in molecular weight has been reported to be higher in MCT2 than in other isoforms, and the 40 kDa value found in pigs is within the limits (40-43 kDa) reported earlier in the literature (Halestrap and Price 1999, Bonen 2000). Anti-MCT4 also resulted in a single band (about 45 kDa) in all tissues studied, with the size similar to that reported earlier (Bonen 2000, Kirk et al. 2000, Manning Fox et al. 2000). For CD147 analysis in the gastrointestinal tract, a commercial anti-mouse antibody was used. In the Western blot, the apparent molecular weight of the band was 110 kDa, which is in accord with previous results and suggests that CD147 forms a complex with MCT1 (Kirk et al. 2000, Buyse et al. 2002). When the samples were stored for a longer period, a 55 kDa band appeared, probably representing a single CD147 protein on the basis of its molecular weight (Buyse et al. 2002).

For HSP72 analysis, a commercial quantitative HSP72 ELISA kit (StressGen Biotechnologies Corp.) was used. The antibody in the kit is anti-mouse HSP72 antibody, but the HSP72 gene is highly conserved; there is 90% similarity between mouse and swine HSP72 sequences (Kiang and Tsokos 1998, Liu and Steinacker 2001, Liu et al. 2006). The same primary antibody has also been used by others in studies of pig HSP72 (Marruchella et al. 2004), and the producer of the kit has confirmed that the antibody is suitable for porcine samples. The antibody has been shown not to react with constitutive HSP73. At the time of the experiments, the test kit was indicated to be suitable for tissue homogenates, serum and plasma. Recently, however, the company has announced that serum and plasma contain proteins that may form complexes with HSP72, thus inducing error in the assay. The latest kit is not recommended for serum or plasma samples (Stressgen Bioreagents Corp., Customer letter November 13, 2006).

In this study EP were stunned by electrical stunning, SP were stunned with CO_2 and wild pigs were shot. Earlier studies have shown that the stunning method may have an impact on many metabolites measured also in this study (Weeding et al. 1993, Hunter et al. 1994). However, the amount of MCT and intracellular HSP cannot be affected by the stunning or slaughter method, because synthesis of new proteins is a slow process in comparison to the time scale of sampling (for example Currie and White 1983, Khassaf et al. 2001, David et al. 2002).

6.2 Selection of muscles for the study

In Study 1 muscles differing from each other in oxidative capacity were selected. These muscles were *M. longissimus dorsi*, *M. semimembranosus*, *M. gluteus superficialis*, *M. masseter* and *M. infraspinatus*. These muscles are also commercially important, with the exception of M. The most oxidative muscles were the M and IS, as indicated by their higher activity of CS and HAD than the LD, G and S, which were strongly glycolytic. Enzyme activities are similar to those previously reported in porcine muscles (Karlström 1995) and consistent with the reported fibre type

composition. Ruusunen and Puolanne (2004) have analysed the fibre type composition of the pigs used in this study, and according to their results, about 80% of muscle fibres in the LD, S and G were fast glycolytic, as opposed to only 20% in IS and 5% in M.

The same muscles were also examined in the wild pigs. Activities of HAD and CS were higher in all five muscles of wild pigs compared to domestic pigs, but LDH activities did not differ significantly. When different muscles of wild pigs were compared, significantly lower activities of HAD and CS were found in LD muscle than in the other four muscles (Table 2). Other muscles did not differ from each other. This result differs from that in domestic pigs, where the more oxidative muscles (IS and M) had higher activities of HAD and CS than the glycolytic muscles (G, S and LD) (Table 2). LD muscle is mainly used in maintaining body balance, whereas G, S and IS muscles are involved in locomotion, which could be one explanation for this phenomenon. The distribution of enzyme activities can be anticipated to be more original in wild pigs, which have not been bred for high total muscle mass like domestic pigs. Although the group of wild pigs in this study was very heterogeneous (e.g. in age, size, genetic background, physical activity), the results are good indicators of overall differences between wild pigs and domestic pigs.

In Study 4 the muscles selected were those used for standard measurements of meat quality in a carcass.

6.3 MCTs in porcine muscles (1)

MCTs are the most important proteins facilitating efflux of lactate and protons from muscles. In Study 1, the average *post-mortem* lactate concentrations in glycolytic muscles were 52-59 mmol/kg wet weight, and in M and IS muscles 30-40 mmol/kg wet weight. Because the samples were taken at slaughter and at least a 15-min delay occurred between death and sampling, the values are much higher than in LD muscle at rest, 11 mmol/kg wet weight (Henckel et al. 2002).

In porcine muscles, the MCT isoform composition differs from that previously reported in humans or rats (Juel and Halestrap 1999, Bonen 2000, Juel 2001), but the composition is similar in the domestic pig and the wild boar. In all five muscles examined, the predominant isoforms were MCT2 and MCT4, whereas MCT1 was found only in small amounts. The MCT4 isoform existed in relatively equal amounts in all muscles, but the content of MCT2 varied according to the oxidative/glycolytic capacity of the muscle in domestic pigs. The amount of MCT4 was higher in the muscles of wild boar than in domestic pigs, but the amounts of MCT1 and MCT2 did not differ significantly.

MCTs transport lactate and protons according to their electrochemical gradient (Juel and Halestrap 1999). Thus, during exercise, when muscles produce lactate, it is transported into plasma, but in most species at rest and during recovery from exercise, red muscles are the major consumers of blood lactate (Gladden 2000). The role of MCT1 in muscles has been suggested to be to transport lactate from plasma to muscle fibres for oxidation (McGullagh et al. 1996, Halestrap and Price 1999, Juel and Halestrap 1999). Very little MCT1 was found in the five porcine muscles investigated. For LD, G and S muscles, this finding is in accord with the notion that MCT1 is virtually indetectable or insignificant in muscles with a high proportion of fast-twitch glycolytic fibres (McGullagh et al. 1996, Wilson et al. 1998). This suggests that porcine muscles do not express MCT1 because of their mitochondrial density, and thus, their capacity to oxidize lactate is low. The reason why the more oxidative porcine muscles M and IS, and the muscles of wild boars also had very low MCT1 content cannot be determined based on these results, however, some

factors regulating gene or protein expression probably prevent the generation of MCT1 in porcine muscles.

MCT2 was found in all five muscles of the pig. This isoform has only recently been detected in the muscles of man, hamsters and rats, while in some other species, e.g. the horse, it is not present (Garcia et al. 1995, Bonen et al. 2006, Koho et al. 2006). Because porcine muscles have low oxidative capacity, the concentration of lactate in these muscles at rest is significantly higher than in the muscles of for instance, horses (Valberg 1989, Henckel et al. 2002). Thus, MCT2 with its low K_m for lactate (Lin et al. 1998, Bröer et al. 1999) could provide a means for pH regulation in the resting muscles of pigs. This notion is supported by the finding that the relative amount of MCT2 was the highest in the most glycolytic muscles, as indicated by the negative correlations between MCT2 and the oxidative enzymes CS and HAD. Although the light muscles of domestic pigs produce small amounts of lactic acid continuously, the resting pH in these muscles is the same as in dark muscles (Kylä-Puhju et al. 2004), which could be the result of the higher quantity and function of MCT2 in light muscles than in dark muscles. Moreover, cancer cell lines, which are known to have very anaerobic metabolism, have been shown to express MCT2 (Lin et al. 1998). However, in addition to the low oxidative capacity, other factors are involved in the regulation of expression of MCT2 since the oxidative muscles M and IS and the muscles of wild boars also expressed MCT2.

MCT4 has been suggested to be the main lactate transporter at high lactate concentrations, which are seen, for example, during intense exercise (Wilson et al. 1998, Bonen et al. 2000, Dimmer et al. 2000, Manning Fox et al. 2000). In pigs, almost equal amounts of MCT4 were found in oxidative M and IS muscles and in the less oxidative muscles LD, G and S. MCT4 in pigs likely has a similar function as in other species. Interestingly, in the muscles of wild boar, the amount of MCT4 was significantly greater than in the same muscles of domestic pigs. In human subjects, both endurance and high intensity training have been shown to increase MCT4 content (Pilegaard et al. 1999a, Dubouchaud et al. 2000), and the difference between the domestic pig and wild boar can possibly be explained by the more active life of the wild boar.

All MCT isoforms studied are capable of facilitating lactate efflux both at rest and during stressful situations, but with a different capacity. It is suggested that at rest, acidification is prevented by MCT2, but because the capacity of MCT2 is saturable due to its low V_{max} value (Lin et al. 1998, Bröer et al. 1999), MCT4 with its high V_{max} (Wilson et al. 1998, Dimmer et al. 2000, Manning Fox et al. 2000) is required to prevent acidification during stress, when the capacity of MCT2 is exceeded.

6.4 MCTs and CD147 in the gastrointestinal tract (2)

The pH of intestinal content, both in the small intestine and in the colon, is usually between 6 and 7 (Bergman 1990). At this pH range, both lactic acid and SCFAs are almost completely ionized, therefore requiring a transporter protein for transcellular absorption. Several mechanisms for absorption have been proposed. Due to Na^+/H^+ exchange, pH is lower in the vicinity of the apical membrane of the epithelium than at the centre of the lumen (Lucas and Blair 1978, Binder and Mehta 1989). This acidic microenvironment increases the percentage of the acid forms of lactate and SCFAs, and enables their diffusion across the membrane. Diffusion alone cannot, however, explain all absorption (Coady et al. 2004); transporters, such as MCTs, are required (Takanaga et al. 1995, Tamai et al. 1995, Ritzhaupt et al. 1998a, 1998b). In addition to MCTs, the non-electrogenic pH-dependent HCO_3^-/monocarboxylate exchange protein (Schröder et al. 2000) and sodium monocarboxylate transporter 1 (SMCT1), which transports small monocarboxylic acids together with Na^+ with a 1:3 stoichiometry between Na^+ and monocarboxylates (Coady et al. 2004, Srinivas

et al. 2005), have been suggested to facilitate the influx of SCFA, at least in the colon. Recently, Srinivas et al. (2005) also found SMCT2 protein in mouse kidney, small intestine and skeletal muscle. In the intestinal tract, SMCT2 is expressed in the proximal parts, whereas SMCT1 is expressed in the distal parts. These locations suggest that the physiological function of SMCT2 is to mediate the absorption of diet-derived SCFA and lactate in the small intestine, and the function of SMCT1 is to mediate the absorption of SCFA derived from bacterial fermentation in the colon (Srinivas et al. 2005).

Substrate specificity of MCTs is broad, and in addition to lactate, MCTs facilitate the transport of many other monocarboxylates, such as SCFAs and ketone bodies (Halestrap and Meredith 2004). Both lactate and SCFAs are formed in the gastrointestinal tract of pigs due to fermentation in the stomach, and additional SCFAs are formed in the colon (Argenzio and Southworth 1974, Bergman 1990). The main SCFAs are acetate, propionate and butyrate (Bergman 1990). Among SCFAs, butyrate has a special role because it is the key substrate for energy production in the epithelial cells of the intestines and it also regulates proliferation of enterocytes (Wächtershäuser and Stein 2000). Recent studies also suggest that butyrate inhibits release of proinflammatory cytokines, such as TNF-α and IL-12, and upregulates the production of anti-inflammatory cytokines, such as IL-10 (Saemann et al. 2000, Nancey et al. 2002). The importance of butyrate was also demonstrated in a feeding study in which food supplemented with sodium n-butyrate increased average daily weight gain of pigs by 23.5% and feed consumption by 8.9% (Gálfi and Bokori 1990). Thus, the transport of SCFAs, especially butyrate, in the epithelial cells of the gut is crucial for the well-being of pigs (Wächtenshäuser and Stein 2000).

In this study, MCT1, MCT2 and MCT4 were found in the samples taken from the small intestine. The isoform profile is similar to that in human subjects (Fishbein et al. 2002), but differs from that in Syrian hamsters, where only MCT4 is expressed (Garcia et al. 1995). In the small intestine of the pig, the main monocarboxylate anion is lactate, formed in the stomach. In the proximal part of the small intestine, lactate concentrations of up to 20 mM have been measured (Argenzio and Southworth 1974; our laboratory, unpublished results), which is well above the K_m of MCT1 (3.5 mM, Bröer et al. 1998) and MCT2 (0.7 mM, Bröer et al. 1999) and within the range of the reported K_m of MCT4 (17-34 mM, Dimmer et al. 2000). Since in the small intestine the efficient absorption of nutrients is of key importance, the expression of the high-affinity isoform, MCT2, can be speculated to be necessary for the complete absorption of lactate. In support of this view, the amount of MCT2 remained unchanged when proximal and distal parts of the small intestine were compared, while the amount of MCT1 decreased towards the distal end of the small intestine.

In the colon, two isoforms, MCT1 and MCT4, were found. This pattern is similar to that in the caecum of Syrian hamsters (Garcia et al. 1995), but differs from the isoform pattern in the human colon, where MCT2 was present (Fishbein et al. 2002). In the porcine colon SCFA concentrations of up to 80-230 mM have been measured (Argenzio and Southworth 1974), which are well above the reported K_m value for butyrate transport in Caco-2 cells (2.6 mM, Hadjiagapiou et al. 2000).

MCT1 and MCT4 attain proper conformation and full activity on cell membranes only in the presence of a chaperone protein, CD147, with which they form a tight complex (Kirk et al. 2000, Buyse et al. 2002, Koho et al. 2002). In this study, CD147 content was significantly higher in Yorkshire pigs than in Landrace pigs. Furthermore, a positive correlation between the amounts of CD147 and MCT1 was found in the small intestine, although CD147 did not show decreased quantities from the proximal to the distal parts of the small intestine, as was the case for MCT1.

MCT1/CD147 ratios were significantly higher in Yorkshire pigs than in Landrace pigs both in the small intestine (3.7 *vs.* 1.5) and in the colon (3.3 *vs.* 1.5). A similar difference has previously been demonstrated in horses (Koho et al. 2002), where horses with an MCT1/CD147 ratio of 3.4 had low lactate transport influx into their red blood cells and horses with an MCT1/CD147 ratio of 0.5 had significantly higher lactate transport activity (0.61 *vs.* 2.75 mmol/mg x min). When the MCT1/CD147 ratios in the intestines of Yorkshire and Landrace pigs are compared with those in equine red blood cells, the question emerges of whether the rate of absorption of monocarboxylates from the chyme varies in these two breeds, and, more importantly, whether the level of MCT and/or CD147 expression in the colon varies sufficiently strongly to influence the rate of butyrate transport, and thus, the welfare of enterocytes.

The breed difference in the amounts of CD147 suggests that several alleles of CD147 are regulated at the level of transcription. In human subjects, three different mutations of MCT1 cDNA have been described and each one of these influences the rate of lactate transport (Merezhinskaya et al. 2000). Single-nucleotide polymorphisms (SNPs) have also been detected in equine MCT1 and CD147, but these polymorphisms apparently have no effect on lactate transport activity (Reeben et al. 2006).

6.5 Porcine HSPs (3)

HSP72 is induced during stress in cells (Hendrick and Hartl 1993, Kiang and Tsokos 1998), where its expression is activated by many pathological and environmental factors (Kiang and Tsokos 1998), but it has also been shown to be expressed in the absence of measurable stress (Tanguay et al. 1988, Beck et al. 1995). HSP72 concentrations at least in skeletal muscle show large interindividual variation (Khassaf et al. 2001), a finding also present in this study when HSP72 concentrations were measured in samples taken from IS and LD muscles, the small intestine and the colon. Schwerin et al. (2001) have suggested that C/A transversion near the GC box (position -232) in the promoter area of the porcine *hsp70.2* gene might explain the variation in HSP72 concentrations.

HSPs are intracellular proteins, but HSP72 has also been found in blood (Romo-Figueroa et al. 1997, Pockley et al. 1998). Febbraio et al. (2002) speculated that during physical exercise the origin is visceral organs such as the liver/intestines. Our results suggest that in pigs HSP72 may be released into the circulation from the intestines because we found a positive correlation between serum and colon HSP72, and also between serum and small intestine HSP72 concentrations. Furthermore, no correlation was present between HSP72 in the liver and serum. Intestinal fatty acid binding protein (I-FABP) is shown to be a good indicator of intestinal epithelial damage, which could also be used to verify these speculations (Niewold et al. 2004), but the concentrations of I-FABP were not measured from these pigs. There are at least two possible explanations for the appearance of HSP72 in serum. First, serum HSP72 levels may reflect changes in the permeability of the intestinal epithelium induced by stress. Second, HSP72 may be actively transported out of the cells by exocytosis, as suggested by Lancaster and Febbraio (2005). Whatever the mechanism for the release of HSP72, it is clear that the porcine intestines become ischaemic upon even short transportation (Nabuurs et al. 2001b), and ischaemia is associated with increased intestinal permeability (Niewold et al. 2000). Thus, it was hypothesized that HSP72 released into blood from stress-sensitive tissues might prove to be a good measure of animal welfare, but the recent complications in the assay method, as discussed above, indicate a need for a more reliable method to measure the concentrations.

In healthy human subjects, HSP72 concentration in serum has been shown to be less than 5 µg/l (Kimura et al. 2004). In the present study on pigs, serum HSP72 concentrations were well below

this limit, but started to rise when colon HSP72 concentration increased to 5.3 μg/g of tissue. This may be a point where colonic epithelium starts to lose its integrity and HSP72 is released into blood, possibly without any induction or synthesis of new HSP72. Because several hours are needed for the induction and synthesis of HSP72, the HSP72 in the circulation at the time of slaughter might have been synthesized in cells before transportation to the slaughterhouse and released from the intestines during transport and slaughter. Thus, if serum HSP72 is used as a stress marker, it is important to consider whether it is the stress of transportation or the stress at the farm that is measured. Additional information of possible long term stress might have been gained by studying a possible correlation between HSP72 and adrenal weight. However, the use of serum or plasma HSP72 as a stress marker would require a pig-specific test kit that is suitable for serum or plasma samples. Such a kit is not currently available, as the producer of the test kit used here has announced that the new antibody lot does not produce repeatable and reliable results for the analysis of serum or plasma samples (Stressgen Bioreagents Corp., Customer letter November 13, 2006). This has been verified in our laboratory.

A correlation between HSP72 in the small intestine or colon and other stress markers was not found. This was not surprising because many of the stress markers used measure short-term stress, whereas HSP72 may be a measure of more chronic stress. Serum HSP72, which can be rapidly released from injured cells, correlated with blood lactate, glucose and FFA, but not with cortisol or haptoglobin. The lack of a correlation between cortisol and HSP72 is in accordance with an earlier study showing that corticosterone or adrenocorticotropic hormone does not play a role in the elevation of extracellular HSP72 during stress (Johnson et al. 2005). The same study also showed that the release of HSP72 is activated by pathway mediated by α-adrenergic receptors, where the most likely ligand is noradrenaline (Johnson et al. 2005).

Chronic stress has been shown to reduce growth (Santos et al. 2000). In the present study, heavier animals had smaller HSP72 concentrations in the colon than smaller animals. Thus, it can be speculated that pigs with a higher concentration of HSP72 were stressed and did not grow as well as less stressed animals, which had lower levels of HSP72. This finding suggests that intracellular/visceral HSP72 could be an indicator of stress. On the other hand, Tsukimi and Okabe (2001) have reported a positive relationship between HSP72 induction and gastric mucosal protection, which seems to contradict our finding of a negative correlation between growth and HSP72. However, they also report that in the small intestine and colon the induction of HSP72 does not necessarily result in mucosal protection. They suggest that the role of different HSPs in gastrointestinal mucosal defence varies depending on the location.

Hessing et al. (1994, 1995) have classified piglets into two groups according to their coping styles during stress. These groups show behavioural, physiological and immunological differences tested in various challenge tests in later life. Their results show that the group of so-called resistant pigs are “active copers”, whereas the group of non-resistant pigs use a more passive coping strategy. It would be interesting to determine whether some differences in these results could also be explained by different coping strategies.

6.6 Hsp70.2 promoter analysis (4)

The same six SNPs in the promoter area of the porcine *hsp70.2* gene found in the Finnish Landrace and Finnish Yorkshire pigs of this study were previously found by Schwerin et al. (1999, 2001) and Chen et al. (2000). The seventh SNP that Chen et al. (2000) identified was outside the area sequenced in this study. The breeds investigated in those studies were Duroc, German and Taiwanese Landrace, Taiwanese Yorkshire, German Large White, Pietrain and German Saddleback.

The allelic frequencies of the polymorphisms show differences between Finnish Landrace and Finnish Yorkshire pigs, but Finnish Landrace pigs also differ in allelic frequencies from Taiwanese Landrace, and Finnish Yorkshires from both German and Taiwanese Yorkshires.

As mentioned above, Schwerin et al. (2001) have reported that C/A transversion at position -232 causes interindividual variation in the expression of porcine HSP72. This transversion influences the binding of transcription factors, and thus, the activity of the gene. In the present study, this could not be confirmed because no differences were present in the expression of HSP72 between different genotypes at position -232. Only pigs with a T/C transition at -118 had slightly higher HSP72 content than animals with 'wild-type' T/T, but no conclusions can be made on the basis of this occasional difference. However, a large variation was present in HSP72 concentrations in LD muscle. The reason for this may be differences in gene activity that are apparent only in situations where animals have experienced stress. In pigs, protein levels have been shown to increase 6 h after the stressful situation (David et al. 2002), and thus, in these pigs the increase in the amount of HSP72 due to transport stress might not have yet been detectable during sampling; duration of the transport to the slaughterhouse together with the stay in the slaughterhouse before exsanguination was less than 6 h. Thus, HSP72 in the LD of these pigs was probably already synthesized at the farm, where some animals likely experienced more stress than others because of, for example, social hierarchies.

In addition to four SNPs, a novel insert (GGAGAGATC) was found at location -11 in Finnish Landrace pigs. The most interesting finding was that in pigs in which the 9-nucleotide insertion was found drip loss seemed to be increased. Unfortunately, drip loss was measured only in EPs, where the number of either homozygous or heterozygous animals was only 14. Because drip loss is a commercially significant meat quality parameter, it would be important to confirm this finding with studies in a larger group of Landrace pigs and also to determine, whether such an insert is also present in breeds other than Finnish Landrace.

6.7 Perspectives

Stress is a significant factor for the welfare of the pig, and furthermore, has adverse effects on the quality of pork (Rosenvold and Andersen 2003). However, measuring stress is not straightforward, and further work is needed to identify suitable markers for long- and short-term stresses. While attempts should be made to minimize stress, animals with the best stress tolerance should be used in breeding. The drawback in this study was that the genetic backgrounds of Finnish pigs are very similar, which could be why many of the physiological parameters measured, such as meat quality, growth and enzyme activities, did not have large variation within the group. Even though interindividual variation between pigs in the amounts of MCTs and HSPs exists, the variation could have been larger had the pigs come from different genetic backgrounds.

MCTs have an important function in skeletal muscle in preventing cellular acidification (Juel 1996, Halestrap and Price 1999), and acidosis of the cell is one of the most important factors triggering HSP72 synthesis (Weitzel et al. 1985). In porcine skeletal muscle, MCTs appear to be well adapted to function in reducing acidity in normal circumstances, as reflected in HSP72 concentrations being low compared with the gastrointestinal tract. However, in the gut, many other stress factors, such as bacteria, viruses and other noxious particles, may be even more important than acidity (Söderholm and Perdue 2001). In human melanoma cells cultured at low extracellular pH (6.7), cell survival is reduced when activity of MCT1 is inhibited (Coss et al. 2003). This leads to acidification of cells and also attenuates their stress response, as indicated by low heat shock-induced expression of HSP72 (Coss et al. 2003). It is tempting to speculate that if this is true also in vivo, continuous

acidification with stress could lead to reduced HSP72 induction. In pigs, this is a normal situation when stressful conditions change the vascular resistance, which results in decreased blood supply in the splanchnic area. This leads easily to ischaemia in the gastrointestinal system (Niewold et al. 2000), which has been shown to be associated with intestinal acidosis in pigs (Ljungdahl et al. 1997). Thus, during stress, it is important that MCT proteins function properly to maintain a sufficiently high intracellular pH for HSP72 production.

During acute stress, lactate concentration is increased in blood and skeletal muscles and is transported out of the muscles mainly by MCT4. With MCT2 in their skeletal muscles, the domestic pig is partially adapted to constant lactate production, despite having otherwise rather unfavourable physiological characteristics, such as large muscle fibre cross-sectional area and poor capillarization. However, when animal stress is discussed, it is also important to determine the applicable indicators of stress and to define the type of stress that they measure. Stress-induced HSP72 seems to have large interindividual variation in pigs, suggesting that it may have potential as a marker of stress in this species.

7. Conclusions

- The muscles of the domestic pig have adapted well to anaerobic metabolism at a molecular level. Both oxidative and glycolytic skeletal muscles express MCT2 and MCT4, which are able to efficiently remove lactate and protons at a wide concentration range. MCT2 may function as the housekeeping lactate transporter, preventing acidification in resting muscles when lactate production is low, and MCT4 facilitates lactate efflux at high lactate concentrations that occur with muscular tension caused by, for instance, stress.
- The activities of citrate synthase and 3-hydroxyacyl-CoA dehydrogenase were significantly higher in wild pigs than in domestic pigs, but when MCTs were compared in these two groups, only the amount of MCT4 was different. More MCT4 was present in the muscles of wild pigs than in domestic pigs.
- No correlation between the amounts of MCTs and any of the meat quality parameters investigated (pH, drip loss, colour) was found.
- At least three MCT isoforms are expressed in the gastrointestinal tract of pigs. MCT1 and MCT4 were found in both the small intestine and the colon, whereas MCT2 was present only in the small intestine, where it may be of key importance in the transport of lactate. The expression pattern of MCT1 and MCT4 suggests that these two isoforms are involved in the absorption of both lactate and SCFA.
- The amount of CD147 was higher in the colon and small intestine of Yorkshire than Landrace pigs. Since CD147 is essential for the activity of MCT1 and MCT4, the breed difference suggests that MCT activity may be higher in Yorkshire than in Landrace pigs.
- Serum HSP72 was correlated with HSP72 in the colon and small intestine, suggesting that the intestines are the origin of serum HSP72. Elevated HSP72 concentrations in serum may reflect changes in the permeability of gut membranes, possibly due to stress caused by transport and handling.
- A negative correlation between the concentration of HSP72 in the colon and carcass weight (growth) was found. As chronic stress attenuates growth, attempts to determine whether the HSP72 content of the colon could be used as an indicator of chronic stress in pigs are warranted.
- The same polymorphisms in the *hsp70.2* gene promoter area were found in Finnish Landrace and Finnish Yorkshire pigs as in other studies, but the allelic frequencies of these

polymorphisms in the Finnish pig breeds differed from those in the corresponding breeds in other countries. In addition, a novel insertion (GGAGAGATC) 11 bp upstream from the start site of translation in Finnish Landrace pigs was discovered.

- Polymorphisms at the promoter area of the *hsp70.2* gene could be connected to the expression of HSP72 in LD only in the case of the T/C transition at -118, but several effects on meat quality were detected. The most interesting of these was that drip loss seems to be increased in those Landrace pigs in which the 9-nucleotide insertion was found. Drip loss is a commercially significant feature in meat quality, and thus, this finding warrants further studies.

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References

Argenzio R.A. and Southworth M. (1974) Sites of organic acid production and absorption in the gastrointestinal tract of the pig. American Journal of Physiology 228: 454-460.

Asea A. (2005) Stress proteins and initiation of immune response: Chaperokine activity of Hsp72. Exercise and Immunology Reviews 11: 34-45.

Bader R. (1983) Vergleichende histometrische und histologische untersuchungen and der skelettmuscular von wild- und hausschweinen, Berlin, München, Tierärzliche Wochenschrift 96: 89-97.

Barton Gade P., Blaabjerg L. and Christensen L. (1995) New low stress system for pig slaughter: Effect on pig behaviour and meat quality. In: Proceedings of the 41st annual International Congress of Meat Science and Technology, San Antonio, Texas, USA. Vol 2: 98-99.

Beck S.C., Paidas C.N., Mooney M.L., Deitch E.A. and De Maio A. (1995) Presence of the stress-inducible form of hsp-70 (hsp-72) in normal rat colon. 3: 398-402.

Becker B.A., Nienaber J.A., Christenson R.K., Manak R.C., DeShazer J.A. and Hahn G.L. (1985) Peripheral concentration of cortisol as an indicator of stress in the pig. American Journal of Veterinary Research 46: 1034-1038.

Bee G., Solomon M.B., Czerwinski S.M., Long C. and Pursel V.G. (1999) Correlation between histochemically assessed fiber type distribution and isomyosin and myosin heavy chain content in porcine skeletal muscles. Journal of Animal Science 77: 2104-2111.

Beecher G.R., Kastenschmidt L.L., Hoekstra W.G., Cassens R.G. and Briskey E.J. (1969) Energy metabolites in red and white striated muscles of the pig. Journal of Agriculture and Food Chemistry 17: 29-33.

Bendall J.R. (1973) Structure and function of muscle. Bourne G.H. (Ed), Academic Press, New York, 243 p.

Bendall J.R., Hallund O. and Wismer-Pedersen J. (1963) Post-mortem changes in muscles of Landrace pigs. Journal of Food Science 28: 156-162.

Bennett E.J., Tennant C.C., Piesse C., Badcock C.-A. and Kellow J.E. (1998) Level of chronic life stress predicts clinical outcome in irritable bowel syndrome. Gut 43: 256-261.

Bergersen L., Waerhaug O., Helm J., Thomas M., Laake P., Davies A.J., Wilson M.C., Halestrap A.P. and Ottersen O.P. (2001) A novel postsynaptic density protein: the monocarboxylate transporter MCT2 is co-localized with delta-glutamate receptors in postsynaptic densities of parallel fiber-Purkinje cell synapses. Experimental Brain Research 136:523-34.

Bergman E.N. (1990) Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiological Reviews 70: 567-583.

Bertschinger H.U. and Nielsen N.O. (1993) Edema disease. In: Diseases of Swine. 6th edition J. Taylor, 498-509.

Binder H.J. and Mehta P. (1989) Short-chain fatty acids stimulate active sodium and chloride absorption in vitro in the rat distal colon. Gastroenterology 96: 989-996.

Bonen A. (2000) Lactate transporters (MCT proteins) in heart and skeletal muscles. Medical Science in Sports Exercise 32: 778-789.

Bonen A., Heynen M. and Hatta H. (2006) Distribution of monocarboxylate transporters MCT1-MCT8 in rat tissues and human skeletal muscle. Applied Physiology, Nutrition, and Metabolism 31: 13-39.

Bonen A., McCullagh K.J., Putman C.T., Hultman E., Jones N.L. and Heigenhauser G.J. (1998) Short-term training increases human muscle MCT1 and femoral venous lactate in relation to muscle lactate. American Journal of Physiology 274:E102-E107.

Bonen A., Miskovic D., Tonouchi M., Lemieux K., Wilson M.C., Marette A. and Halestrap A.P. (2000) Abundance and subcellular distribution of MCT1 and MCT4 in heart and fast-twitch skeletal muscles. American Journal of Physiology (Endocrinology and Metabolism) 278: E1067-E1077.

Borthakur A., Gill R.K., Hodges K., Ramaswamy K., Hecht G. and Dudeja P.K. (2006) Enteropathogenic Escherichia coli inhibits butyrate uptake in Caco-2 cells by altering the apical membrane MCT1 level. American Journal of Physiology (Gastrointestinal Liver Physiology) 290: G30-G35.

Bottoms G.D., Roesel O.F., Rausch F.D. and Akins E.L. (1972) Circadian variation in plasma cortisol and corticosterone in pigs and mares. American Journal of Veterinary Research 33: 785-790.

Bracke M.B.M. and Hopster H. (2006) Assessing the importance of natural behavior for animal welfare. Journal of Agricultural and Environmental Ethics 19: 77-89.

Branscheid W. (1998) Consumer demands on meat between price and ethics. Deutsche Tierarztliche Wochenschrift 105: 289-292.

Bröer S., Bröer A., Schneider H.-P., Stegen C., Halestrap A.P. and Deitmer J.W. (1999) Characterization of the high-affinity monocarboxylate transporter MCT2 in *Xenopus laevis* oocytes. Biochemical Journal 341: 529-535.

Bröer S., Schneider H.-P., Bröer A., Rahman B., Hamprecht B. and Deitmer J.W. (1998) Characterization of the monocarboxylate transporter 1 expressed in *Xenopus laevis* oocytes by changes in cytosolic pH. Biochemical Journal 333: 167-174.

Buyse M., Sitaraman S.V., Liu X., Bado A. and Merlin D. (2002) Luminal leptin enhances CD147/MCT-1-mediated uptake of butyrate in the human intestinal cell line Caco2-BBE. Journal of Biological Chemistry 31: 28182-28190.

Campsi J., Leem T.H. and Fleshner M. (2003) Stress-induced extracellular Hsp72 is a functionally significant danger signal to the immune system. Cell Stress & Chaperones 8: 272-286.

Chen M.Y., Huang S.Y., Tsou H.L., Lin E.C., Yang P.C., Kuo Y.H., Huang T.Y. and Lee W.C. (2000) Polymorphism in the 5'-flanking region of porcine heat shock protein 70.2 gene. Animal Genetics 31: 404-419.

Chronin G.M., Wiepkema P.R. and van Ree J.M. (1986) Experientia 42: 198.

Coady M.J., Chang M.-H., Charron F.M., Plata C., Wallendorff B., Sah J.F., Markowitz S.D., Romero M.F. and Lapointe J.-Y. (2004) The human tumour suppressor gene *SLC5A8* expresses a Na^+-monocarboxylate cotransporter. Journal of Physiology 557: 719-731.

Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology: Recommended method for the determination of four enzymes in blood (1974) Scandinavian Journal of Clinical and Laboratory Investigation 338: 291-306.

Coss R.A., Storck C.W., Daskalakis C., Berd D. and Wahl M.L. (2003) Intracellular acidification abrogates the heat shock response and compromises survival of human melanoma cells. Molecular Cancer Therapeutics 2: 383-388.

Cuff M.A., Lambert D.W. and Shirazi-Beechey S.P. (2002) Substrate-induced regulation of the human colonic monocarboxylate transporter, MCT1. Journal of Physiology 539: 361-371.

Currie R.W. and White F.P. (1983) Characterization of the synthesis and accumulation of a 71-kilodalton protein induced in rat tissues after hyperthermia. Canadian Journal of Biochemistry and Cell Biology 61: 438-446.

Daly K., Cuff M.A., Fung F. and Shirazi-Beechey S.P. (2005) The importance of colonic butyrate transport to the regulation of genes associated with colonic tissue homoeostasis. Biochemical Society Transitions 33: 733-735.

Dammrick K. (1970) Polyarthrosis in fattened pigs as a constitution-dependent breeding disease. Berliner und Munchener tierarztliche Wochenschrift 15: 450-456.

David J.C., Grongnet J. and Lalles J.P. (2002) Weaning affects the expression of heat shock proteins in different regions of the gastrointestinal tract of piglets. Journal of Nutrition 132: 2551-2561.

Dimmer K.S., Friedrich B., Lang F., Deitmer J.W. and Bröer S. (2000) The low-affinity monocarboxylate transporter MCT4 is adapted to the export of lactate in highly glycolytic cells. Biochemical Journal 350: 219-227.

Doizé F., Laporte R. and Deroth L. (1989) Effects of exercise on skeletal muscle and serum enzyme activities in pigs. Veterinary Research Communications 13: 341-347.

Doornenbal H. and Tong A.K.W. (1981) Growth, development and chemical composition of the pig. 4. Relative growth of visceral organs. Growth 45: 279-285.

Dubouchaud H., Butterfield G.E., Wolfel E.E., Bergman B.C. and Brooks G.A. (2000) Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle. American Journal of Physiology (Endocrinology and Metabolism) 278: E571-E579.

Eckersall P.D. (2004) Recent advances in the use of acute phase proteins as biomarkers for infection and inflammation in veterinary clinical biochemistry. In: Proceedings of the XI Congress International Society of Animal Clinical Biochemistry, Valdivia, Chile. 59-62.

Enerson B.E. and Drewes L.R. (2003) Molecular features, regulation, and function or monocarboxylate transporters: implications for drug delivery. Journal of Pharmaceutical Sciences 92: 1531-1544.

Essén-Gustavsson B. (1986) Activity and inactivity-related muscle adaptation in the animal kingdom. Saltin, B. (ed.): Biochemistry of Exercise VI. Human Kinetic Publishers, Campaigne, IL, 435-444.

Essén-Gustavsson B. and Fjelkner-Modig S. (1985) Skeletal muscle characteristics in different breeds of pigs in relation to sensory properities of meat. Meat Science 13: 33-47.

Essén-Gustavsson B. and Jensen-Waern M. (1993) Muscle characteristics and metabolic response at slaughter in domestic pigs reared either outdoors or indoors. In: Proceedings of the 39th International Congress of Meat Science and Technology, Calgary, Canada. S2P09.WP.

Essén-Gustavsson B., Karlström K. and Lundström K. (1988) Muscle metabolism and glycogen depletion within fibre types at slaughter in pigs with different halothane genotypes. Relation to meat quality properties. In: Proceedings of 34th International Congress of Meat Science and Technology, Brisbane, Australia. Part B: 581-583.

Fanelli A., Grollman E.F., Wang D. and Philip N.J. (2003) MCT1 and its accessory protein CD147 are differentially regulated by TSH in rat thyroid cells. American Journal of Physiology (Endocrinology and Metabolism) 285: E1223-E1229.

Febbraio M.A., Ott P., Bay Nielsen H., Steensberg A., Keller C., Krustrup P., Secher N.H. and Klarlund Pedersen B. (2002) Exercise induces hepatosplanchnic release of heat shock protein 72 in humans. Journal of Physiology 544.3: 957-962.

Fishbein W.N., Merezhinskaya N. and Foellmer J.W. (2002) Relative distribution of three major lactate transporters in frozen human tissues and their localization in unfixed skeletal muscle. Muscle & Nerve 26: 101-112.

Fitts R.H., Nagle F.J. and Cassens R.G. (1973) Characteristics of skeletal muscle fiber types in the miniature pig and the effect of training. Canadian Journal of Physiology and Pharmacology 51: 825-831.

Fjelkner-Modig S. (1985) Sensory and biophysical properties of pork. Academic dissertation. University of Lund, Sweden. -139 p.

Flanagan S.W., Ryan A.J., Gisolfi C.V. and Moseley P.L. (1995) Tissue-specific HSP70 response in animals undergoing heat stress. American Journal of Physiology (Regulatory, Integrative and Comparative Physiology) 37: R28-R32.

Franck M., Monin G., Figwer P., Poirel M.T. and Legault C. (2002). Strukturloses Fleisch – ein ernsthaftes Problem. Problematik bei der industriellen Herstellung von Kochschinken. Fleischwirtschaft 12: 97-100.

Friesema E.C.H., Ganguly S., Abdalla A., Manning-Fox J.E., Halestrap A.P. and Visser T.J. (2003) Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. Journal of Biological Chemistry. 278: 40128-40135.

Froberg M.K., Gerhart D.Z., Enerson B.E., Manivel C., Guzman-Paz M., Seacotte N. and Drewes L.R. (2001) Expression of monocarboxylate transporter MCT1 in normal and neoplastic human CNS tissues. Neuroreport 12:761-765.

Fukudo S., Abe K., Hongo M., Utsumi A. and Itoyama Y. (1997) Brain-gut induction of heat shock protein (HSP) 70 mRNA by psychophysiological stress in rats. Brain Research 757: 146-148.

Gálfi P. and Bokori J. (1990) Feeding trial in pigs with a diet containing sodium n-butyrate. Acta Veterinaria Hungarica 38: 3-17.

Garcia C.K., Brown M.S., Pathak R.K. and Goldstein J.L. (1995) cDNA cloning of MCT2, a second monocarboxylate transporter expressed in different cells than MCT1. Journal of Biological Chemistry 270: 1843-1849.

Gerhart D.Z., Enerson B.E., Zhdankina O.Y., Leino R.L. and Drewes L.R. (1998) Expression of the monocarboxylate transporter MCT2 by rat brain glia. Glia 22: 272-281.

Gladden B.L. (2000) Muscle as a consumer of lactate. Medicine & Science in Sports & Exercise 32: 764-771.

Gottesmann P. and Hamm R. (1986) Methode zur unterscheidung der zwerchfellmusculatur (Saumfleish) von der übrigen skelettmuskulatur des rindes. Fleischwirtschaft 66: 1267-1270.

Green H., Halestrap A., Mockett C., O'Toole D., Grant S. and Ouyang J. (2002) Increases in muscle MCT are associated with reductions in muscle lactate after a single exercise session in humans. American Journal of Physiology (Endocrinology and Metabolism) 282: E154-E160.

Gregory N.G. (1998) Animal welfare and meat science. Wallingford, UK: CAB International, 298 p.

Gunther E. and Walter L. (1994) Genetic aspects of the hsp70 multigene family in vertebrates. Experientia 50: 987-1001.

Gutierrez J.A. and Guerriero Jr V. (1995) Chemical modifications of a recombinant bovine stress-inducible 70 kDa heat-shock protein (Hsp70) mimics Hsp70 isoforms from tissues. Biochemical Journal 305: 197-203.

Hadjiagapiou C., Schmidt L., Dudeja P.K., Layden T.J. and Ramaswamy K. (2000) Mechanism(s) of butyrate transport in Caco-2 cells: role of monocarboxylate transporter 1. American Journal of Physiology (Gastrointestinal Liver Physiology) 279: G775-G780.

Halestrap A.P. and Meredith D. (2004) The SLC16 gene family - from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. Pflügers Archiv – European Journal of Physiology 447: 619-628.

Halestrap A.P. and Price N.T. (1999) The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation. Biochemical Journal 343: 281-299.

Henckel P., Karlsson A., Jensen M.T., Oksbjerg N. and Petersen J.S. (2002) Metabolic conditions in porcine *longissimus* muscle immediately preslaughter and its influence on peri- and post mortem energy metabolism. Meat Science 62: 145-155.

Hendrick J.P. and Hartl F.-U. (1993) Molecular chaperone functions of heat-shock proteins. Annual Review of Biochemistry 62: 349-384.

Hessing M.J.C., Coenen G.J., Vaiman M. and Renard C. (1995) Individual differences in cell-mediated and humoral immunity in pigs. Veterinary Immunology and Immunopathology 41: 97-113.

Hessing M.J.C., Hagelso A.M., van Beek J.A.M., Wiepkema P.R. and Schouten W.G.P. (1994) Individual behavioral and physiological strategies in pigs. Physiology & Behavior 55: 39-46.

Hofmann K. (1990) Definition and measurement of meat quality. In: Proceedings of 36th International Congress of Meat Science and Technology, Havana, Cuba. vol 3: 941-954.

Hood D.E. and Riordan E.B. (1973) Discoloration in pre-packed beef. Journal of Food Technology 8: 333.

Huff-Lonergan E., Mitsuhashi T., Beekman D.D., Parrish Jr. D.G., Olson D.G. and Robson R.M. (1996) Proteolysis of specific muscle structural proteins by μ-calpain at low pH and temperature is similar to degradation in postmortem bovine muscle. Journal of Animal Science 74: 993-1008.

Hunter E.J., Weeding C.M., Guise H.J., Abbott T.A. and Penny R.H.C. (1994) Pig welfare and carcase quality: a comparison of the influence of slaughter handling systems at two abattoirs. Veterinary Record 135: 423-425.

Isosaki M. and Nakashima T. (1998) Psychological stress induces heat shock protein 70 expression in rat aorta. Japanese Journal of Pharmacology 76: 305-308.

Jackson V.N., Price N.T., Carpenter L. and Halestrap A.P. (1997) Cloning of the monocarboxylate transporter isoform MCT2 from rat testis provides evidence that expression in tissues is species-specific and may involve post-translational regulation. Biochemical Journal 324: 447-453.

Jackson V.N., Price N.T. and Halestrap A.P. (1995) cDNA cloning of MCT1, a monocarboxylate transporter from rat skeletal muscle. Biochimica et Biophysica Acta 1238: 193-196.

Janssens C.J.J.G., Helmond F.A. and Wiegant V.M. (1994) Increased cortisol response to exogenous adrenocorticotropic hormone in chronically stressed pigs: influence of housing conditions. Journal of Animal Science 72: 1771-1777.

Janssens C.J.G., Houwing H., Helmond F.A. and Wiegant V.M. (1993) The influence of chronic stress on cortisol, prolactin, and reproductive hormones during the oestrus cycle of the pig. Pflügers Archive, Journal of European Physiology 424: R5 (abstract).

Jensen-Waern M. and Nyberg L. (1993) Valuable indicators of physical stress in porcine plasma. Zentralblatt für Veterinarmedizin. Reihe A 40: 321-327.

Jóhannsson E., Lunde P.K., Heddle C., Sjaastad I., Thomas M.J., Bergersen L., Halestrap A.P., Blackstad T.W., Ottersen O.P. and Sejersted O.M. (2001) Upregulation of the cardiac monocarboxylate transporter MCT1 in a rat model of congestive heart failure. Circulation 104: 729-734.

Johnson J.D., Campisi J., Sharkey C.M., Kennedy S.L., Nickerson M. and Fleshner M. (2005) Adrenergic receptors mediate stress-induced elevations in extracellular Hsp72. Journal of Applied Physiology 99: 1789-1795.

Johnstone R.M. (2005) Revisiting the road to the discovery of exosomes. Blood Cells, Molecules, and Diseases 34: 214-219.

Jorgensen P. and Hyldgaard-Jensen J.F. (1975) The effect of physical training on skeletal muscle enzyme composition in pigs. Acta Veterinaria Scandinavica 16: 368-378.

Juel C. (1996) Lactate/proton co-transport in skeletal muscle: regulation and importance for pH homeostasis. Acta Physiologica Scandinavica 156: 369-374.

Juel C. (1998) Muscle pH regulation: role of training. Acta Physiologica Scandinavica 162: 359-366.

Juel C. (2001) Current aspects of lactate exchange: lactate/H^+ transport in human skeletal muscle. European Journal of Applied Physiology 86: 12-16.

Juel C. and Halestrap A.P. (1999) Lactate transport in skeletal muscle – role and regulation of the monocarboxylate transporter. Journal of Physiology 517: 633-642.

Juga J., Maijala K., Mäki-Tanila A., Mäntysaari E., Ojala M. and Syväjärvi J. (1999) Kotieläinjalostus. Suomen kotieläinjalostusosuuskunta. Gummerus kirjapaino, Jyväskylä. 294 pp.

Kangasniemi R. (1993) Lihan värinmittaus kantakokeissa muuttuu. Sika 2: 46-48.

Karlsson A. (1993) Porcine muscle fibres- biochemical and histochemical properties in relation to meat quality. Academic dissertation. Swedish University of Agricultural Sciences, Sweden. -49 p.

Karlsson A., Essén-Gustavsson B. and Lundström K. (1994) Muscle glycogen depletion pattern in halothane-gene-free pigs at slaughter and relation to meat quality. Meat Science 38: 91–101.

Karlström K. (1995) Fibre type distribution, capillarization and enzymatic profile of locomotor and nonlocomotor muscles of wild boar and domestic pig. Academic dissertation. Swedish University of Agricultural Sciences, Sweden. -80 p.

Kauffman R.G., Sybesma W., Smulders F.J.M., Eikelonboom G., Engel B., van Laack R.L.J.M., Hoving-Bolink A.H., Sterrenburg P., Nordheim E.V., Walstra P. and van der Wal P. (1993) The effectiveness of examining early post-mortem musculature to predict ultimate pork quality. Meat Science 34: 283-300.

Khassaf M., Child R.B., McArdle A., Brodie D., Esanu C. and Jackson M.J. (2001) Time course of responses of human skeletal muscle to oxidative stress induced by nondamaging exercise. Journal of Applied Physiology 90: 1031-1035.

Kiang J.G. and Tsokos G.C. (1998) Heat Shock Protein 70 kDa: Molecular Biology, Biochemistry, and Physiology. Pharmacology and Therapeutics 80: 183-201.

Kiessling K.-H., Lundström K., Petersson H. and Stålhammar H. (1982) Age and feed related changes of fibre composition in pig muscle. Swedish Journal of Agricultural Research 12: 69-75.

Kimura F., Itoh H., Ambiru S., Shimizu H., Togawa A., Yoshidome H., Ohtsuka M., Shimamura F., Kato A., Nukui Y. and Miyazaki M. (2004) Circulating heat-shock protein 70 is associated with postoperative infection and organ dysfunction after liver resection. American Journal of Surgery 187: 777-784.

Kirat D., Inoue H., Iwana H., Hirayama K., Yokota H., Taniyama H. and Kato S. (2005) Expression and distribution of monocarboxylate transporter 1 (MCT1) in the gastrointestinal tract of calves. Research in Veterinary Science 79: 45-50.

Kirat D., Inoue H., Iwana H., Hirayama K., Yokota H., Taniyama H. and Kato S. (2006) Monocarbosylate transporter 1 gene expression in the ovine gastrointestinal tract. Veterinary Journal 171: 462-467.

Kirk P., Wilson M.C., Heddle C., Brown M.H., Barclay A.N. and Halestrap A.P. (2000) CD147 is tightly associated with lactate transporters MCT1 and MCT4 and facilitates their cell surface expression. EMBO Journal 19: 3896-3904.

Knott R.M., Robertson M., Muckersie E., Folefac V.A., Fairhurst F.E., Wileman S.M. and Forrester J.V. (1999) A model system for the study of human retinal angiogenesis: activation of monocytes and endothelial cells and the association with the expression of the monocarboxylate transporter type 1 (MCT-1). Diabetologia 42: 870-877.

Koho N.M., Hyyppä S. and Pösö A.R. (2006) Monocarboxylate transporters (MCT) as lactate carriers in equine muscle and red blood cells. Equine Veterinary Journal, Supplement 36: 354-358.

Koho N., Maijala V., Norberg H., Nieminen M. and Pösö A.R. (2005) Expression of MCT1, MCT2 and MCT4 in the rumen, small intestine and liver of reindeer (*Rangifer tarandus tarandus* L.). Comparative Biochemistry and Physiology, Part A 141: 29-34.

Koho N.M., Väihkönen L.K. and Pösö A.R. (2002) Lactate transport in red blood cells by monocarboxylate transporters. Equine Veterinary Journal, Supplement 34: 555-559.

Kylä-Puhju M., Ruusunen M., Kivikari R. and Puolanne E. (2004) The buffering capasity of porcine muscles. Meat Science 67: 587-593.

Ladewig J., de Passillé A.M., Rushen J., Schouten W., Terlouw E.M.C. and von Borell E. (1993) Stress and physiological correlates of stereotypic behaviour. In: Stereotypic Animal Behaviour: Fundamentals and Applications to Welfare (eds. Lawrence A.B. and Rushen J.). CAB International, Trowbridge, 97-118.

Lancaster G.I. and Febbraio M.A. (2005) Mechanisms of stress-induced cellular HSP72 release: implications for exercise-induced increases in extracellular HSP72. Exercise and Immunology Reviews 11: 46-52.

Lefaucheur L., Ecolan P., Plantard L. and Guefuen N. (2002) New insights into muscle fiber types in the pig. The Journal of Histochemistry and Cytochemistry 50: 719-730.

Leino R.L., Gerhart D.Z., Duelli R., Enerson B.E. and Drewes L.R. (2001) Diet-induced ketosis increases monocarboxylate transporter (MCT1) levels in rat brain. Neurochemistry International 38: 519-527.

Lepore D.A., Knight K.R., Anderson R.L. and Morrison W.A. (2001) Role of priming stress and Hsp70 in protection from ischemia-reperfusion injury in cardiac and skeletal muscle. Cell Stress & Chaperones 6: 93-96.

Lin R.-Y., Vera J.C., Chaganti R.S.K. and Golde D.W. (1998) Human monocarboxylate transporter 2 (MCT2) is a high affinity pyruvate transporter. Journal of Biological Chemistry 273: 28959-28965.

Lindfors L., Berg C. and Algers B. (2005) Integration of natural behavior in housing systems. Ambio 34: 325-330.

Lindholm A., Persson S., Jonsson L. and Andrén H. (1979) Biochemical and histochemical properties and fibre distribution of muscles in trained, untrained and halothane sensitive pigs. Acta Agricultural Scandinavica, Supplement 22: 357-363.

Liu Y., Gampert L., Nething K. and Steinacker J.M. (2006) Response and function of skeletal muscle heat shock protein 70. Frontiers in Bioscience 11: 2802-2827.

Liu Y. and Steinacker J.M. (2001) Changes in skeletal muscle heat shock proteins: pathological significance. Frontiers in Bioscience 6: d12-25.

Ljungdahl M., Rasmussen I., Raab Y., Hillered L. and Haglund U. (1997) Small intestinal mucosal pH and lactate production during experimental ischemia-reperfusion and fecal peritonitis in pigs. Shock 2: 131-138.

Locke M. (1997) The cellular stress response to exercise: role of stress proteins. Exercise and Sport Science Reviews 25: 105-136.

Lucas M.L. and Blair J.A. (1978) The magnitude and distribution of the acid microclimate in proximal jejunum and its relation to luminal acidification. Proceedings of the Royal Society of London, B 200: 27-41.

Lucke J.N., Hall G.M. and Lister D. (1977) Anaesthesia of pigs sensitive to malignant hyperthermia. Veterinary Record 100: 45-48.

Madshus I.H. (1988) Regulation of intracellular pH in eukaryotic cells. Biochemical Journal 250: 1-8.

Manning Fox J.E., Meredith D. and Halestrap A.P. (2000) Characterization of human monocarboxylate transporter 4 substantiates its role in lactic acid efflux from skeletal muscle. Journal of Physiology 529: 285-293.

Marple D.N. and Cassens R.G. (1973) Increased metabolic clearance of cortisol by stress-susceptible swine. Journal of Animal Science 36: 1139-1142.

Marruchella G., Di Leonardo M., Di Guardo M., Romanucci M., Marà M., Tiscar P.G., Mosca F. and Della Salda, L. (2004) Heat shock proteins (HSPs) 27, 72 and 73 in normal and pre-ulcerative mucosa of the gastric pars oesophagea in swine. Journal of Comparative Pathology 131: 10-17.

Martí O., Martí J. and Armario A. (1994) Effects of chronic stress on food intake in rats: influence of stressor intensity and duration of daily exposure. Physiology & Behavior 55: 747-753.

McAllister R.M., Reiter B.L., Amann J.F. and Laughlin M.H. (1997) Skeletal muscle biochemical adaptations to exercise training in miniature swine. Journal of Applied Physiology 82: 1862-1868.

McGullagh K.J.A., Poole R.C., Halestrap A.P., O'Brien M. and Bonen A. (1996) Role of the lactate transporter (MCT1) in skeletal muscles. American Journal of Physiology (Endocrinology and Metabolism) 271: E143-E150.

McKiman M.D., Gray C.G. and White F.C. (1991) Effects of feeding on muscle blood flow during prolonged exercise in miniature swine. Journal of Applied Physiology 70: 1097-1104.

Merezhinskaya N., Fishbein W.N., Davis J.I. and Foellmer W. (2000) Mutations in MCT1 cDNA in patients with symptomatic deficiency in lactate transport. Muscle & Nerve 23: 90-97.

Miller S.A., Dykes D.D. and Polesky H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 16: 1215.

Million M., Taché Y. and Anton P. (1999) Susceptibility of Lewis and Fischer rats to stress-induced worsening of TNB-colitis: protective role of brain CRF. American Journal of Physiology (Gastrointestinal and Liver Physiology) 276: G1027-G1036.

Minvielle B., Le Strat P., Lebret B., Houix Y., Boulard J. and Clochefert N. (2001). Viandes déstructurées. Situation dans cinq abattoirs de l'Ouest de la France: facteurs de risque et proposition d'un modèle. Caractérisation colorimétrique, biochimique et histologique. Journées Recherche Porcine en France 33: 95-101.

Monin G. and Sellier P. (1985) Pork of low technological quality with a normal rate of muscle pH fall in the immediate post-mortem period: The case of the Hampshire breed. Meat Science 13: 49-63.

Morimoto R.I., Jurivich D.A., Kroeger P.E., Mathur S.K., Murphy S.P., Nakain A., Sarge K., Abravaya K. and Sistonen L.T. (1994) Regulation of heat shock gene transcription by a family of heat shock factors. The Biology of Heat Shock Proteins and Molecular Chaperones. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 417-455.

Moseley P.L., Gapen C., Wallen E.S., Walter M.E. and Peterson M.W. (1994) Thermal stress induces epithelial permeability. American Journal of Physiology (Cell Physiology) 267: C425-C434.

Moseley P.L., Wallen E.S., McCafferty J.D., Flanagan S. and Kern J.A. (1993) Heat stress regulates the human 70-kDa heat shock gene through the 3' – untranslated region. American Journal of Physiology (Lung Cellular and Molecular Physiology) 264: L533-L537.

Murray C.M., Hutchinson R., Bantick J.R., Belfield G.P., Benjamin A.D., Brazma D., Bundick R.V., Cook I.D., Craggs R.I., Edwards S., Evans L.R., Harrison R., Holness E., Jackson A.P., Jackson C.G., Kingston L.P., Perry M.W., Ross A.R., Rugman P.A., Sidhu S.S., Sullivan M., Taylor-Fishwick D.A., Walker P.C., Whitehead Y.M., Wilkinson D.J., Wright A. and Donald D.K. (2005) Monocarboxylate transporter MCT1 is a target for immunosuppression. Nature Chemical Biology 1:371-376.

Müller F., Huber K., Pfannkuche H., Aschenbach J.R., Breves G. and Gäbel G. (2002) Transport of ketone bodies and lactate in the sheep ruminal epithelium by monocarboxylate transporter 1. American Journal of Physiology (Gastrointestinal Liver Physiology) 283: G1139-G1146.

Nabuurs M.J.A., van de Weijgert E.J.H.M., Grootendorst A.F. and Niewold T.A. (2001a) Oedema disease is associated with metabolic acidosis and small intestinal acidosis. Research in Veterinary Science 70: 247-253.

Nabuurs M.J.A., van Essen G.J., Nabuurs P., Niewold T.A. and van der Meulen, J. (2001b) Thirty minutes transport causes small intestinal acidosis in pigs. Research in Veterinary Science 70: 123-127.

Nancey S., Bienvenu J., Coffin B., Andre F., Descos L. and Flourie B. (2002) Butyrate strongly inhibits in vitro stimulated release of cytokines in blood. Digestive Diseases and Sciences 47: 921-928.

Negrão A.B., Deuster P.A., Gold P.W., Singh A. and Chrousos G.P. (2000) Individual reactivity and physiology of the stress response. Biomedicine & Pharmacotherapy 54: 122-128.

Niewold T.A., Meinen M. and van der Meulen J. (2004) Plasma intestinal fatty acid binding protein (I-FABP) concentrations increase following intestinal ischemia in pigs. Research in Veterinary Science 77: 89-91.

Niewold T.A., van Essen G.J., Nabuurs M.J.A., Stockhofezurwieden N. and van der Meulen J. (2000) A review of porcine pathophysiology: a different approach to disease. Veterinary Quarterly 22: 209-212.

Okamura H., Spicer S.S. and Schulte B.A. (2001) Developmental expression of monocarboxylate transporter in the gerbil inner ear. Neuroscience 107: 499-505.

Orsenigo M.N., Tosco M., Bazzini C., Laforenza U. and Faelli A. (1999) A monocarboxylate transporter MCT1 is located at the basolateral pole of rat jejunum. Experimental Physiology 84: 1033-1042.

Otten W., Kanitz E., Puppe B., Tuchscherer M., Brussow K.P., Nurnberg G. and Stabenow B. (2004) Acute and long term effects of chronic intermittent noise stress on hypothalamic-pituitary-adrenocortical and sympatho-adrenomedullary axis in pigs. Animal Science 78: 271-283.

Papp E., Nardai G., Soti C. and Csermely P. (2003) Molecular chaperones, stress proteins and redox homeostasis. BioFactors 17: 249-257.

Petersen H.H., Nielsen J.P. and Heegaard P.M.H. (2004) Application of acute phase protein measurements in veterinary clinical chemistry. Veterinary Research 35: 163-187.

Petersen J.S., Henckel P., Maribo H., Oksbjerg N. and Sørensen M.T. (1997) Muscle metabolic traits, post mortem-pH-decline and meat quality in pigs subjected to regular physical training and spontaneous activity. Meat Science 46: 259-275.

Pilegaard H., Domino K., Noland T., Juel C., Hellsten Y., Halestrap A.P. and Bangsbo J. (1999a) Effect of high-intensity exercise training on lactate/H^+ transport capacity in human skeletal muscle. American Journal of Physiology (Endocrinology and Metabolism) 276: E255-E261.

Pilegaard H., Terzis G., Halestrap A.P. and Juel C. (1999b) Distribution of the lactate/H^+ transporter isoforms MCT1 and MCT4 in human skeletal muscle. American Journal of Physiology (Endocrinology and Metabolism) 276: E843-E848.

Pittet J.-F., Lee H., Morabito D., Howard M.B., Welch W.J. and Mackersie R.C. (2002) Serum levels of Hsp 72 measured early after trauma correlate with survival. Journal of Trauma 52: 611-617.

Plastow G.S., Carrión D., Gil M., García-Regueiro J.A., Font i Furnols M., Gispert M., Oliver M.A., Guàrdia M.D., Hortós M., Rius M.A., Sàrraga C., Díaz I., Valero A., Sosnicki A., Klont R., Dornan S., Wilkinson J.M., Evans G., Sargent C., Davey G., Connolly D., Houeix B., Maltin C.M., Hayes H.E., Anandavijayan V., Foury A., Geverink N., Cairns M., Tilley R.E., Mormède P. and Blott S.C. (2005) Quality of pork genes and meat production. Meat Science 70: 409-421.

Plourde V. (1999) Stress-induced changes in the gastrointestinal motor system. Canadian Journal of Gastroenterology, Supplement A 13: 26A-31A.

Pockley A.G., Shepherd J. and Corton J.M. (1998) Detection of heat shock protein 70(HSP70) and anti-HSP70 antibodies in the serum of normal individuals. Immunological Investigations 27: 367-377.

Poole R.C. and Halestrap A.P. (1993) Transport of lactate and other monocarboxylates across mammalian plasma membranes. American Journal of Physiology (Cell Physiology) 264: C761-C782.

Poole R.C., Sansom C.E. and Halestrap A.P. (1996) Studies of the membrane topology of the rat erythrocyte H^+/lactate cotransporter (MCT1). Biochemical Journal 320: 817-824.

Price N.T., Jackson V.N. and Halestrap A.P. (1998) Cloning and sequencing of four new mammalian monocarboxylate transporter (MCT) homologues confirms the existence of a transporter family with an ancient past. Biochemical Journal 320: 755-759.

Py G., Lambert K., Perez-Martin A., Raynaud E., Prefaut C. and Mercier J. (2001) Impaired sarcolemmal vesicle lactate uptake and skeletal muscle MCT1 and MCT4 expression in obese Zucker rats. American Journal of Physiology (Endocrinology and Metabolism) 281: E1308-E1315.

Pösö A.R., Eklund-Uusitalo S., Hyyppä S. and Pirilä E. (2002) Induction of heat shock protein 72 mRNA in skeletal muscle by exercise and training. Equine Veterinary Journal, Supplement 34: 214-218.

Pösö A.R. and Jensen-Waern M. (1992) Does a single bout of exercise cause adaptation of amino acid metabolism in pigs? Research in Veterinary Sciences 53: 331-337.

Qiu B.S., Vallance B.A., Blennerhassett P.A. and Collins S.M. (1999) The role of CD4+ lymphocytes in the susceptibility of mice to stress-induced reactivation of experimental colitis. Nature Medicine 5: 1178-1182.

Rea I.M., McNerlan S. and Pockley A.G. (2001) Serum heat shock protein and anti-heat shock protein antibody levels in aging. Experimental Gerontology 36: 341-352.

Reeben M., Koho N.M., Raekallio M., Hyyppä S. and Pösö A.R. (2006) MCT1 and CD147 gene polymorphisms in Standardbred horses. Equine Veterinary Journal, Supplement 36: 322-325.

Reiland S. (1978) The effect of decreased growth rate on frequency and severity of osteochondrosis in pigs. Acta Radiologica 358: 107-122.

Renerre M. (1990) Review: factors involved in the discoloration of beef meat. International Journal of Food Science and Technology 25: 613-630.

Ritzhaupt A., Elli A., Hosie K.B. and Shirazi-Beechey S.P. (1998a) The characterization of butyrate transport across pig and human colonic luminal membrane. Journal of Physiology 507: 819-830.

Ritzhaupt A., Wood I.S., Ellis A., Hosie K.B. and Shirazi-Beechey S.P. (1998b) Identification and characterization of a monocarboxylate transporter (MCT1) in pig and human colon: its potential to transport L-lactate as well as butyrate. Journal of Physiology 513: 719-732.

Romo-Figueroa M.G., Camou J.P. and Yepiz-Plascencia G.M. (1997) Stress-70 proteins in plasma from stressed and unstressed pigs. Journal of Food Biochemistry 21: 67-78.

Roozen A.W., Tsuma V.T. and Magnusson U. (1995) Effects of short term restraint stress on plasma concentrations of catecholamines, beta-endorphin, and cortisol in gilts. American Journal of Veterinary Research 56: 1225-1227.

Rosenvold K. and Andersen H.J. (2003) Factors significance for pork quality. Meat Science 64: 219-237.

Ruusunen M. (1994) Muscle histochemical properties of different pig breeds in relation to meat quality. Academic dissertation. University of Helsinki, Finland. -77 p.

Ruusunen M., Partanen K., Pösö R. and Puolanne E. (2007) The effect of dieatary protein supply on carcass composition, size of organs, muscle properties and meat quality of pigs. Livestock Science 107: 170-181.

Ruusunen M. and Puolanne E. (1997) Comparison of histochemical properties of different pig breeds. Meat Science 45: 119-125.

Ruusunen M. and Puolanne E. (2004) Histochemical properties of fibre types in muscles of wild and domestic pigs and the effect of growth rate on muscle fibre properties. Meat Science 67: 533-539.

Ruusunen M., Sevon-Aimonen M.-L. and Puolanne E. (1996) Composition and cross-sectional area of muscle fibre types in relation to daily gain and lean and fat content of carcass in Landrace and Yorkshire pigs. Agricultural and Food Science in Finland 5: 593-600.

Saemann M.D., Bohmig G.A., Osterreicher C.H., Burtscher H., Parolini O., Diakos C., Stockl J., Horl W.H. and Zlabinger G.J. (2000) Anti-inflammatory effects of sodium butyrate on human monocytes: potent inhibition of IL-12 and up-regulation of IL-10 production. FASEB Journal 14: 2380-2382.

Salzman A.L., Wang H., Wollert P.S., Vandermeer T.J., Compton C.C., Denenberg A.G. and Fink M.P. (1994) Endotoxin-induced ileal mucosal hyperpermeability in pigs: role of tissue acidosis. American Journal of Physiology (Gastrointestinal Liver Physiology) 266: G633-G646.

Santos J., Benjamin M., Yang P.C., Prior T. and Perdue M.H. (2000) Chronic stress impairs rat growth and jejunal epithelial barrier function: Role of mast cells. American Journal of Physiology (Gastrointestinal Liver Physiology) 278: G847-G854.

Scheppach W. (1994) Effects of short chain fatty acids on gut morphology and function. Gut, Supplement 1: S35-S38.

Scheppach W., Sommer H., Kirchner T., Paganelli G.-M., Bartram P., Christl S., Richter F., Dusel G. and Kasper H. (1992) Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. Gastroenterology 103: 51-56.

Schrøder-Petersen D.L. and Simonsen H.B. (2001) Tail biting in pigs. Veterinary Journal 162: 196-210.

Schröder O., Opritz J. and Stein J. (2000) Substrate and inhibitor specificity of butyrate uptake in apical membrane vesicles of the rat distal colon. Digestion 62: 152-158.

Schwerin M., Hagendorf A., Fürbass R. and Teuscher F. (1999) The inducible stress protein 70.2 gene - A candidate gene for stress susceptibility in swine. Archives für Tierzuht 42: 61-66.

Schwerin M., Maak S., Hagendorf A., von Lengerken G. and Seyfert H.M. (2002) A 3'-UTR variant of the inducible porcine hsp70.2 gene affects mRNA stability. Biochimica et Biophysica Acta 1578: 90-94.

Schwerin M., Maak S., Kalbe C. and Fuerbass R. (2001) Functional promoter variants of highly conserved inducible *hsp70* genes significantly affect stress response. Biochimica et Biophysica Acta 1522: 108-111.

Sellier P., Mejenes-Quijani A., Marinova P., Talmant A., Jacquet B. and Monin G. (1988) Meat quality as influenced by halothane sensitivity and ultimate pH in three porcine breeds. Livestock Production Science 18: 171-186.

Selye H. (1950) Stress and the general adaptation syndrome. British Medical Journal, June 17: 1383-1392.

Shimizu S., Tani Y., Yamada H., Tabata M. and Murachi T. (1980) Enzymatic determination of serum-free fatty acids: a colorimetric method. Analytical Biochemistry 107: 193-198.

Sjaastad Ø.V., Hove K. and Sand O. (2003) Physiology of domestic animals. Scandinavian Veterinary Press, Oslo, 735 pp.

Solomon M.B., Caperna T.J., Mroz R.J. and Steele N.C. (1994) Influence of dietary protein and recombinant porcine somatotropin administration in young pigs: III. Muscle fiber morphology and shear force. Journal of Animal Science 72: 615-621.

Sorger P.K. and Pelham H.R.B. (1987) Cloning and expression of a gene encoding HSC73, the major HSP70- like protein in unstressed rat cells. EMBO Journal 6: 993-998.

Spencer G.S. (1980) Relationship between plasma somatomedin activity and levels of cortisol and free fatty acids following stress in pigs. Journal of Endocrinology 84: 109-114.

Srere P.A. (1969) Citrate synthase. In: Colowick, S.P. & Kaplan, N.O. (eds), Methods in enzymology, vol. 13: 3-5. Academic Press, New York.

Srinivas S.R., Gopal E., Zhuang L., Itagaki S., Martin P.M., Fei Y.-J., Ganapathy V. and Prasad P.D. (2005) Cloning and functional identification of slc5a12 as a sodium-coupled low-affinity transporter for monocarboxylates (SMCT2). Biochemical Journal 392: 655-664.

Stein J., Zores M. and Schröder O. (2000) Short-chain fatty acid (SCFA) uptake into Caco-2 cells by a pH-dependent and carrier mediated transport mechanism. European Journal of Nutrition 39: 121-125.

Subjeck J. and Shyy T.T. (1986) Stress protein systems of mammalian cells. American Journal of Physiology (Cell Physiology) 250: C1-C17.

Suuronen T. (1995) The relationship of oxidative and glycolytic capacity of Longissimus Dorsi muscle to meat quality when different pig breeds and crossbreeds are compared. Academic dissertation. University of Jyväskylä, Finland. -112 p.

Szentkuti L. and Schlegel O. (1981) Genetische und funktionelle Einflüsse auf Fasertypenanteile und Faserdurchmesser im M. longissimus dorsi und M. semitendinosus von Schweinen. Untersuchungen an trainierten Haus- und immobil gehaltenen Wildschweinen. Deutsche Tierarztliche Wochenschrift 92: 93-97.

Söderholm J.D. and Perdue M.H. (2001) Stress and the gastrointestinal tract II. Stress and intestinal barrier function. American Journal of Physiology (Gastrointestinal Liver Physiology) 280: G7-G13.

Takanaga H., Tamai I., Inaba S., Sai Y., Higashida H., Yamamoto H. and Tsuji A. (1995) cDNA cloning and functional characterization of rat intestinal monocarboxylate transporter. Biochemical and Biophysical Research Communications 217: 370-377.

Tamai I., Takanaga H., Maeda H., Sai Y., Ogihara T., Higashida H. and Tsuji A. (1995) Participation of a proton-cotransporter, MCT1, in the intestinal transport of monocarboxylic acids. Biochemical and Biophysical Research Communications 214: 482-489.

Tanguay R.M. (1988) Transcriptional activation of heat-shock genes in eukaryotes. Biochemistry & Cell Biology 66: 584-593.

Toole B.P. (2003) Emmprin (CD147), a cell surface regulator of matrix metalloproteinase production and function. Current Topics in Developmental Biology 54: 371-389.

Trinder P. (1969) Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of Clinical Biochemistry 6: 24-27.

Tsukimi Y. and Okabe S. (2001) Recent advances in gastrointestinal pathophysiology: role of heat shock proteins in mucosal defense and ulcer healing. Biological & Pharmaceutical Bulletin 24: 1-9.

Valberg S., Essén Gustavsson B., Lindholm A. and Persson S.G.B. (1989) Blood chemistry and skeletal muscle metabolic responses during and after different speeds and durations of trotting. Equine Veterinary Journal 21: 91-95.

van der Wal P.G., Engel B. and Reimert H.G.M. (1999) The effect of stress, applied immediately before stunning, on pork quality. Meat Science 53: 101-106.

van Laack R.L.J.M., Faustman C. and Sebranek J.G. (1993) Pork quality and the expression of stress protein Hsp 70 in swine. Journal of Animal Science 71: 2958-2964.

Voutila L., Mullen A.M., Allen P., Troy D. and Puolanne E. (2005) Thermal stability of connective tissue and meat quality of loose structured pork semimembranosus muscles. In: Proceedings of 51st International Congress of Meat Science and Technology, Baltimoore, Maryland, USA. 228-232.

Walsh R.C., Koukoulas I., Garnham A., Moseley P.L., Hargreaves M. and Febbraio M.A. (2001) Exercise increases serum Hsp72 in humans. Cell Stress & Chaperones 6: 386-393.

Wang Y., Tonouchi M., Miskovic D., Hatta H. and Bonen A. (2003) T_3 increases lactate transport and the expression of MCT4, but not MCT1, in rat skeletal muscle. American Journal of Physiology (Endocrinology and Metabolism) 285: E622-E628.

Warriss P.D., Brown S.N., Edwards J.E., Anil M.H. and Fordham D.P. (1992) Veterinary Record 131: 194.

Weeding C.M., Hunter E.J., Guise H.J. and Penny R.H.C. (1993) Effects of abattoir and slaughter handling systems on stress indicators in pig blood. Veterinary Record 133: 10-13.

Weitzel G., Pilatus U. and Rensing, L. (1985) Similar dose response of heat shock protein synthesis and intracellular pH change in yeast. Experimental Cell Research 159: 252-256.

Welch W.J. (1992) Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and disease. Physiological Reviews 72: 1063-1080.

Wilson M., Jackson V.N., Heddle C., Price N.T., Pilegaard H., Juel C., Bonen A., Montgomery I., Hutter O.F. and Halestrap A.P. (1998) Lactic acid efflux from white skeletal muscle is catalyzed by the monocarboxylate transporter isoform MCT3. Journal of Biological Chemistry 273: 15920-15926.

Wilson M.C., Meredith D. and Halestrap A.P. (2002) Fluorescence resonance energy transfer studies on the interaction between the lactate transporter MCT1 and CD147 provide information on the topology and stoichiometry of the complex *in situ*. Journal of Biological Chemistry 277: 3666-3672.

Wilson M.C., Meredith D., Manning Fox J.E., Manoharan C., Davies A.J. and Halestrap A.P. (2005) Basigin (CD147) is the target for organomercurial inhibition of monocarboxylate transporter isoforms 1 and 4: the ancillary protein for the insensitive MCT2 in embigin (gp70). Journal of Biological Chemistry 280: 27213-27221.

Wright B.H., Corton J.M., El-Nahas A.M., Wood R.F.M. and Pockley A.G. (2000) Eletvated levels of circulating heat shock protein 70 (Hsp70) in peripheral and renal vascular disease. Heart Vessels 15: 18-22.

Wächtershäuser A. and Stein J. (2000) Rationale for the luminal provision of butyrate in intestinal diseases. European Journal of Nutrition 39: 164-171.

Yang T.S. and Lin J.H. (1997) Variation of heart size and its correlation with growth performance and vascular space in domestic pigs. Journal of Animal Science 64: 523-528.

Zhu J., Quyymi A.A., Wu H., Csako G., Rott D., Zalles-Ganley A., Ogunmakinwa J., Halcox J. and Epstein S.E. (2003) Increased serum levels of heat shock protein 70 are associated with low risk of coronary artery disease. Arteriosclerosis, Thrombosis and Vascular Biology 23: 1055-1059.